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No. 43

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GENETICS

UDC: 576.852.18.095.5

ISOLATION OF PLASMID DNA FROM STREPTOMYCES OLIVACEUS V KX

Kiev MIKROBIOLOGICHESKIY ZHURNAL in Russian No 3, 1978 pp 309-314

[Article by T. D. Dekhtyarenko, B. P. Matselyukh and L. F. Boin, Institute of Microbiology and Virology, Ukrainian Academy of Sciences, submitted 30 Nov 77]

[Text] In a previous study, genetic (high incidence of elimination of the antibiotic-synthesizing character) and biochemical (presence of a second band of DNA in a cesium chloride--ethidium bromide density gradient) evidence was obtained of the possibility of plasmid control of antibiotic synthesis and sporulation in *S. olivaceus* V KX [1].

Our objective here was to develop a method of obtaining plasmid DNA from *S. olivaceus* V KX.

Material and Methods

Strains: We used the wild prototrophic strain *S. olivaceus* V KX and auxotrophs, obtained from it, varying in antibiotic synthesizing capacity: 1435 and 1435-32 ant, 24-14 and 24-14 ant, 1049, 1049EB4 ant, 1049 AF ant, 1049-7NG ant, 1049 pil-1 ant, 6 his rec, 6EB ant, 2692, 52-132B, 52-132 ant, 196, 175 ant, as well as the following strains of *S. coelicolor* A3(2) varying in fertility: IF (wild strain) and 585 ade v 10 mth B2 cys D 18 UF, obtained from Hopwood.

Media: Complete medium [2] was used to cultivate the streptomycetes. A saccharose-casamino-glycine medium [3] was used to accumulate mycelium, from which plasmid DNA was isolated.

Production of mycelium and lysis thereof: Mycelium was cultivated by the method of submerged growth on rockers, in saccharose-casamino-glycine medium at 28-30°C for 40-70 h; it was collected by centrifugation and eluted in TES buffer (0.03 M Tris, 0.005 M EDTA, 0.05 M NaCl, pH 8.0). For lysis, we used no more than 2 g mycelium, which was suspended in 15-20 ml 20 or 34% saccharose in TE buffer (0.01 M Tris, 0.001 M EDTA, pH 8.0) and treated with lysozyme using the second variant of the Schrempf method [1],

i.e., we added 0.2 ml 0.2 M EDTA, pH 8.0, and 0.2 ml lysozyme solution (25 or 50 mg in 0.01 M tris-HCl buffer, pH 8.0 per ml cell suspension), with incubation for 5-10 min at 30°C. The mixture was thoroughly precipitated in ice; 0.2 ml 0.25 M EDTA, pH 8.0, and sodium dodecylsulfate to a 1% concentration or other detergents were added: sarcosyl [4], triton X-100 [5], bridge-58 [6].

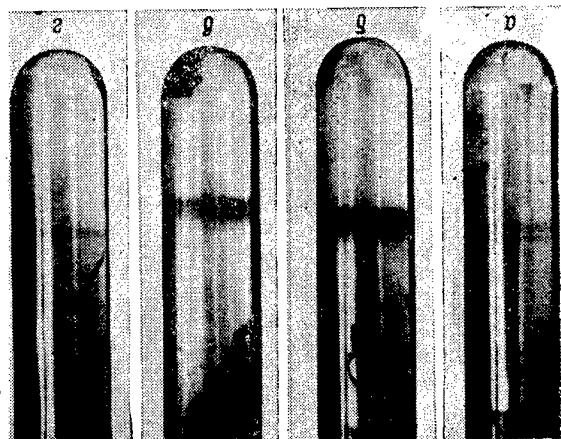


Figure 1.

Stained DNA zones after centrifuging preparations in a cesium chloride--ethidium bromide density gradient.

Strains:

- α) 52-132B
- β) 1435-32
- γ) 1049
- ε) 1049 AF

Cleared lysates were obtained by precipitation of the bulk of chromosomal DNA by means of addition of sodium chloride to a concentration of 1 M and centrifugation of lysates at 28,000 r/min for 20-40 min at 4°C, using a VAC-60 centrifuge.

Production of circular supercoiled DNA: The second variant of the method of Schrempf [3] with various modifications was used to obtain plasmid DNA. We used polyethylene glycol 6000 (PEG) [7] to concentrate supercoiled DNA from cleared lysates. In addition, the cleared lysates were treated with phenol saturated with 0.25 M EDTA, pH 8.4, and DNA from the supernatant was precipitated in ethanol, diluted in SSC buffer (0.15 M NaCl and 0.015 M Na citrate, pH 7.2) and tested for presence of plasmid DNA.

Centrifugation in cesium chloride--ethidium bromide density gradient: Equilibrated centrifuging of DNA was performed at 42,000 r/min for 36-43 h at 2-4°C, on a Beckman L3-50 centrifuge (Ti-50 rotor) or K-32 preparative ultracentrifuge (U-65 rotor). The contents of the test tubes were separated by means of an excavator. Fractions from the stained zones of plasmid DNA were dialyzed against 500 volumes of 0.01 M tris-HCl buffer containing 0.05 M NaCl, pH 7.5, for 2 days, with buffer changes. The density of the fractions was determined by refractometry [8]. In some cases, only the stained zone of the gradient was collected.

Electron microscopy: DNA from the stained gradient zones was dialyzed against 0.15 M ammonia acetate, pH 7.2, and prepared for electron microscopy

after dilution by the protein film method of Kleinschmidt and Zahn [9]. The preparations were examined under a JEM-7 electron microscope at an accelerating voltage of 80 kV and instrument magnification of 13,000-28,000 $\times$ .

## Results

Mycelium cultivated on minimum medium with phenylalanine, described by Schrempf [3], as well as on medium S with glycine [10], was not lysed by the methods at our disposal. Mycelium collected after cultivation on saccharos rose-casamino-glycine medium, with caseine hydrolysate in concentrations of 0.1, 0.5, 1.2 and 2.4% underwent 16, 30-40, 55-63 and 85-100% lysis, respectively. The percentage of cell lysis was determined spectrophotometrically, according to absorption in UV [ultraviolet light] at 260 nm following centrifugation of the lytic mixture. Cell lysis (after cultivation of cells on a medium with 2.4% casein hydrolysate) by lysozyme (produced by the Koch-Light Company) in a concentration of 25 mg/ml was considered 100%. We used lysozyme from the Olaynskiy Chemical Reagent Plant in a concentration of 50 mg/ml.

Use of a 20% solution of saccharose instead of 34% did not affect protoplast formation. The younger, 40-h culture was subject to lysis better than the 70-h culture.

Protoplasts obtained after treating mycelium with lysozyme were submitted to further lysis by means of various detergents: triton X-100, bridge-58, sarcosyl and sodium dodecylsulfate. After addition to protoplasts of an aliquot of triton mixutre or bridge mixture, lysis thereof was observed only after incubation at 37°C for 10 min. Sarcosyl solution, added to a concentration of 0.8-1%, lysed the protoplasts under refrigeration, with aeration using a pipette. The best lysis of mycelium was observed with the use of sarcosyl and sodium dodecylsulfate, which we subsequently used for isolation of plasmid DNA.

Centrifugation of DNA preparations obtained after treatment of cleared mycelial lysates with polyethylene glycol demonstrated the presence of distinct stained bands situated in a cesium chloride-ethidium bromide density gradient of 1.61-1.63 g/cm<sup>3</sup>, with the exception of material from strain 1049 pil-1, the band of which was in the density gradient of 1.64-1.66 g/cm<sup>3</sup> [1]. Plasmid DNA was demonstrated in some strains in the form of a second band in a lower position (Figure 1a) or bottom layer of a single wide band (Figure 1b and c). Evidently, the width of the band is attributable to the heterogeneity of insufficiently separated DNA molecules, represented, as shown by electron microscopy, by molecules of chromosomal DNA (top layer), open circular molecules (middle layer) and supercoiled molecules of plasmid DNA (bottom layer).

Several variants of application on the cesium chloride--ethidium bromide gradient were used for strains 1048, 1049 pil-1, 1435 and 1435-32 ant:  
a) cleared lysate, b) dissolved DNA isolated from cleared lysates with PEG,  
c) DNA obtained using the second variant (b) but treated with nuclease-free

pronase (600 mg/ml, 30 min at 37°C), d) DNA isolated using the second variant (b) but treated with DNAase (50 mg/ml in 10<sup>-3</sup> M MgCl<sub>2</sub>, 30 min at 37°C, e) uncleared lysates treated with pronase under the same conditions, f) the same lysates, but cleared, g) uncleared lysates successively treated with pronase, then cleared with sodium chloride, and polyethylene-glycol-precipitated DNA was applied, after dissolution, to the gradient.

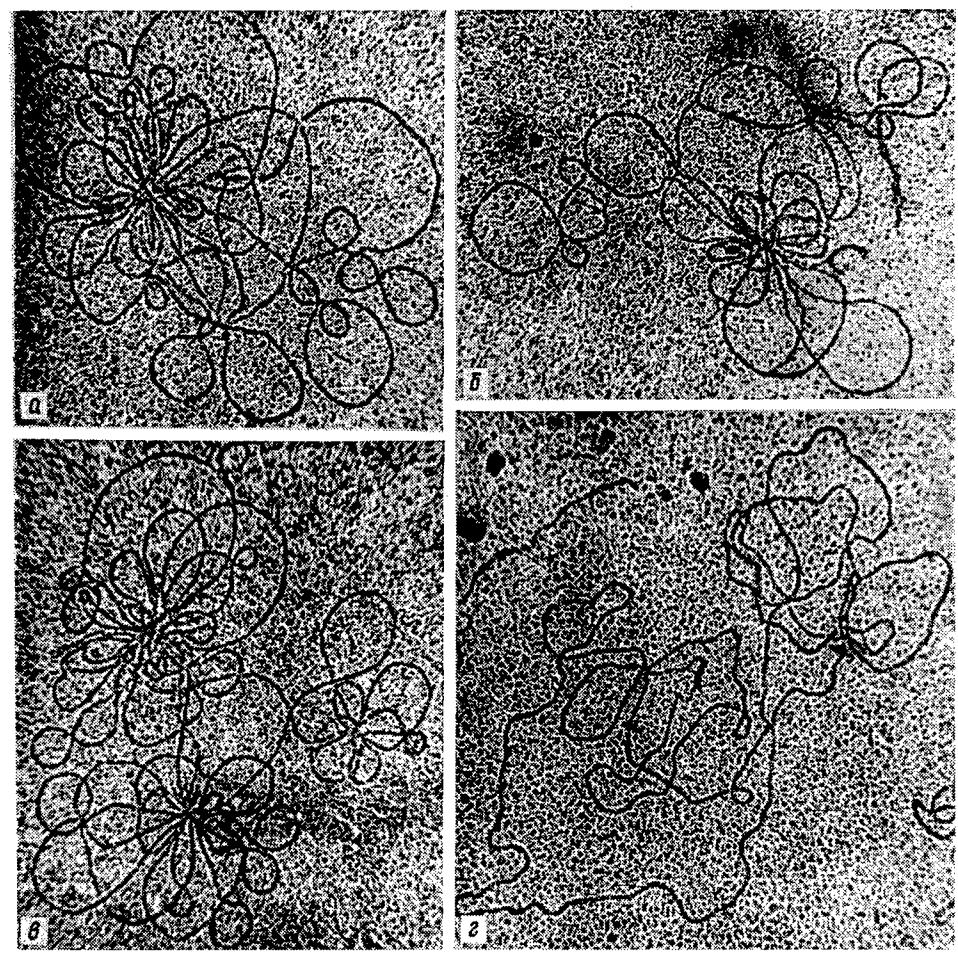


Figure 2. Electronograms of plasmid DNA molecules. Supercoiled molecules in the form of rosettes (13,200 $\times$ ):

a) 16 $\mu$ m	e) 30 $\mu$ m
b) 15 $\mu$ m	f) molecule in the form of an open ring (15 $\mu$ m)

We were unable to obtain a distinct zone of plasmid DNA after centrifugation of cleared lysate (variant a), probably because of an inadequate concentration of the former. Treatment with DNAase (variant d) resulted in disappearance of the zone of plasmid DNA and significant constriction of the zone of

chromosomal DNA. After treatment of uncleared lysates with pronase, regardless of subsequent manipulations, there were diffuse zones of chromosomal and plasmid DNA (variants e, f and g). Two zones were demonstrable in DNA precipitated from PEG-cleared lysates (variant b) and in the same DNA treated with pronase (variant c), and they consisted of chromosomal and plasmid DNA, respectively.

Ethidium bromide was removed from the fractions of plasmid DNA by means of dialysis or repeated extraction with isopropyl alcohol.

The yield of plasmid DNA after dialysis or isopropyl alcohol treatment was virtually the same, constituting 7.1 and 8.2  $\mu$ g for *S. coelicolor* 585, 7.1 and 8.7  $\mu$ g/g wet mycelium for strain *S. olivaceus*.

Electron microscopy of molecules of the plasmid DNA we isolated revealed that they are in the shape of rosettes without free ends, and their contour length constitutes a mean of 15-16  $\mu$ m (Figure 2a and 6). In addition to molecules 15-16  $\mu$ m in size, we encountered some that were 30  $\mu$ m in length (Figure 2e), which are apparently dimers. Treatment of mycelial lysates or polyethylene glycole concentrates of plasmid DNA with pronase changed the rosettes into open circular DNA molecules (Figure 2e).

The presence of plasmid DNA in strains of various origin corresponds to the antibiotic-synthesizing character (see Table).

Characteristics of strains of *S. olivaceus* V KX

Strain	Antibiotic synthesis	Plasmid DNA	Origin
V KX	++	+	Wild type
1049	++	+	UV (from V KX)
1049-NG7 ant	-	-	Nitrosoguanidine (from 1049)
1049 AF ant	-	-	Acriflavine (from 1049)
1049 EB4 ant	-	-	Ethidium bromide (from 1049)
1049 pil-1 ant	-	-	Na dodecylsulfate (from 1049)
1435	++	+	Nitrosoguanidine (from VKX)
1435-32 ant	-	+	UV (from 1435)
24-14	++	+	UV, nitrosoguanidine (from V KX)
24-14 ant	-	-	Spontaneous (from 24-14)
51-132 B	++	+	Spontaneous (from 52-132)
52-132 ant	-	-	Spontaneous (from 52-132B)
2692	++	+	Nitrosoguanidine (from V KX)
2692 bld-1	+	-	Na dodecylsulfate (from 2692)
175 ant	-	-	Spontaneous (from 175)

Note: "+" and +-+ signs refer to presence and absence of the character, respectively.

Elimination of the plasmid by means of acriflavine (Figure 12), ethidium bromide, sodium dodecylsulfate, or spontaneous loss thereof, led to loss of the antibiotic-synthesizing character [1].

Strain 1435-32 is an exception to this rule, since it does not synthesize antibiotic and, at the same time, contains plasmid DNA. Since this strain was obtained under the influence of large doses of UV, it may be assumed that the loss of the property of antibiotic synthesis is not related to plasmid elimination, but to mutation of genes of the latter that determine antibiotic synthesis.

#### Discussion

In this work we continued the study of plasmid DNA previously isolated from some strains of *S. olivaceus* V KX [1]. A comparative evaluation was made of the efficacy of different methods of mycelium lysis, used to obtain bacterial plasmid DNA. It was shown that sarcosyl is the most effective detergent for lysis of protoplasts under refrigeration. The use of this detergent resulted in a better yield of plasmid DNA than the use of sodium dodecylsulfate. Triton X-100 and bridge-58, which did induce lysis of protoplast at 37°C, were not effective in obtaining plasmid DNA from the streptomycetes strains studied.

The use of polyethylene glycol 6000, which permits concentration of DNA from cleared lysates, is an important factor in preparative isolation of unlabeled plasmid DNA from streptomycetes.

The use of sarcosyl and PEG made it possible to isolate, for the first time in this study, plasmid DNA that determines antibiotic synthesis in *S. olivaceus* V KX.

It was previously demonstrated, using genetic techniques, that synthesis of the antibiotic, methylenomycin A, by *S. coelicolor* A3(2) is controlled by the SCPl plasmid [11]; however, repeated attempts at isolating the latter were unsuccessful [3, 12].

The isolated molecules of plasmid DNA are rosette-shaped, i.e., they are in a supercoiled form. Since the rosettes are changed into open rings after pronase treatment, it may be assume that they are a complex of DNA with protein, which was named the relaxation complex.

The contour length of the molecules of plasmid DNA is about 15-16  $\mu\text{m}$ , which corresponds to a molecular weight of  $30-32 \cdot 10^6$ . According to its size, this plasmid is referable to the large plasmids.

The number of copies of plasmid DNA molecules per chromosomes has not yet been precisely determined. According to the preliminary data, plasmid DNA constitutes about 1-2% of all cellular DNA, and it is apparently represented by a small number of copies. It is transmissible, and it performs the function of an effective sex factor, which will be the subject of another report.

## Conclusions

1. Various methods were tested for the lysis of streptomycetal mycelium in order to obtain plasmid DNA. Sarcosyl and, to a lesser extent, sodium dodecyl-sulfate lyze protoplasts more effectively than triton X-100 and bridge-58, and for this reason they can be used with success to isolate plasmid DNA from the mycelium of streptomycetes.
2. For the first time, supercoiled plasmid DNA was isolated from actinomycetes by means of centrifugation in a cesium chloride--ethidium bromide density gradient of total DNA precipitated from cleared mycelial lysates with polyethylene glycol; this plasmid DNA is in the form of rosettes, it has a molecular weight of about  $30-32 \cdot 10^6$  and determines antibiotic synthesis in *S. olivaceus* V KX.

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INDUSTRIAL MICROBIOLOGY

UDC: 582.288-11

FEED YEAST OBTAINED FROM HYDROLYSATES OF PLANT WASTE AND METHANOL

Kiev MIKROBIOLOGICHESKIY ZHURNAL in Russian No 3, 1978 pp 293-299

[Article by V. F. Semenov, V. S. Podgorskiy and V. N. Ivanov, Institute of Microbiology and Virology, Ukrainian Academy of Sciences, submitted 16 Nov 77]

[Text] Of the many problems facing mankind, that of nutrition is one of the most acute. According to the data of FAO [Food and Agriculture Organization, WHO] and UN, at the present time one-third of mankind is experiencing a deficiency of calories in the diet and almost half, a protein deficiency [1]. Primarily the developing countries are experiencing an acute protein shortage. This is due primarily to social causes.

In the Soviet Union, plant protein requirements of the public are fully met; however, the requirements referable to protein of animal origin are not fully met as yet [2]. Production of animal protein by means of microbiological synthesis is an important recourse to compensate for the shortage of this protein and, in the future, it may be the main one.

At the present time, production of protein and vitamin concentrates (BVK) in our country is based on carbohydrate (waste from the food industry, wood pulp and agricultural waste, hydrolysates, sulfite liquor) and hydrocarbon raw material (liquid purified petroleum paraffins). The resources of carbohydrate raw material that is used cannot provide for a sharp increase in BVK production in the future, so that the problem of microbiological synthesis of protein can be resolved only by means of increasing use of abundant and available hydrocarbons of petroleum and natural gas. In the next few years, liquid, purified petroleum paraffins will serve as the principal raw material for microbiological synthesis of protein [3]. At the same time, intensive research on the use of other types of raw material for biosynthesis is being pursued in our country and abroad. Among them, diverse waste from agriculture and methyl alcohol are important.

Hydrolysates of agricultural waste: The problem of processing polysaccharides of plant tissue into monosaccharides and production, on their basis, of certain food and feed products is acquiring increasing importance. The particularly important role of this problem is attributable to the possibility of industrial production of feed protein from plant material with the

use of microorganisms. At the present time, sunflower shells, corn cobs and the husk of grain crops constitute the plant raw material used in the Ukraine in production of feed yeast. Other types of plant raw material are not used in the microbiological industry because of their specific distinctions and lack of developed technology for processing thereof, as well as lack of machinery and mechanisms to gather raw material and prepare it for production.

Estimates show that the resources referable to the traditionally used plant raw material will not provide for continuous operation of the required number of hydrolysis and yeast plants. Work is being conducted at the Institute of Microbiology and Virology, Ukrainian Academy of Sciences, to find new types of nonalimentary plant waste and develop technologies for producing feed yeast from them at hydrolysis and yeast plants. At the present time, over 15 million tons of agricultural waste in this republic are not being put to rational use.

Chemical composition of hydrolysates obtained from agricultural waste

Hydrolysate components, %	Raw material for hydrolysates					
	flax & hemp fiber	rice straw	sun-flower stalks	grape vines	wheat straw	spent tanbark
Reducing substances	1,25	1,34	1,82	2,57	1,52	1,27
Furfural	0,024	0,011	0,014	0,06	0,021	0,036
Brominatable substances	0,036	0,068	0,12	0,112	0,12	0,12
Colloid substances	0,364	0,195	0,449	0,329	0,204	1,626
Organic acids	0,385	0,44	0,3	0,84	0,182	
Sugar constituents:						
glucose	45,2	14,6	27,9	44,5	13,3	15,9
arabinose	20,5	18,9	15,5	8,0	11,6	7,6
xylose	32,2	63,6	40,4	34,2	66,4	67,0
rhamnose	2,0		5,1	4,2		
galactose		2,7	10,0	6,8	8,5	9,3

Research was conducted at the Institute of Microbiology and Virology, Ukrainian Academy of Sciences, on obtaining feed yeast from hydrolysates of rice and wheat straw, sunflower stalks, grapevine cuttings, spent tanbark, flax and hemp fiber.

Chromatographic analysis defined the qualitative composition of hydrolysate sugars. The levels of decomposition products of carbohydrates, which inhibit yeast growth, have been determined (see Table). It was established that it is possible, in principle, to raise yeast on these hydrolysates. A selection was made of yeast cultures that grow actively on hydrolysates; studies have been made of dynamics of their growth and utilization of reducing substances and organic acids; determination was made of the economic effectiveness of the process of yeast cultivation; studies have also been made of the effect of B group vitamins on their growth.

yeast was cultivated in flasks on rockers and laboratory fermenters with automatic regulation of pH and temperature in a continuous process. Missing mineral components were added to the nutrient medium in preparing hydrolysates for yeast cultivation.

In the research, cultures of the following yeasts were used: *Candida tropicalis* K-41, *Candida* sp. K-19-73, *C. scottii* Tul-1, *C. humicola* H-1, *C. blankii* H-3, *C. tenuis* H-6, *Trichosporon* sp. and others.

Among the yeasts isolated from industrial associations and collection cultures, selection was made of highly productive strains, which are stable in hydrolytic media, with a high growth rate under conditions that were similar to industrial ones. *C. humicola* H-1, *Candida* sp. K-19-73, *C. blankii* H-3 and *C. tenuis* H-6 had these properties.

Industrial experiments dealing with production of feed yeast on the above hydrolysates, which were conducted at the Nikolayevskiy Yeast and Hydrolysis Plant, revealed that *C. humicola* H-1 and *Candida* sp. K-19-73 were the predominant cultures. The yield of yeast from specified reducing agents constitutes 48-52%.

The obtained yeast contains 52-58% crude protein, 42-49% protein, 30-33% carbohydrates, 5-6% lipids and ash. The protein contains all the essential amino acids. Lysine content is up to 5%, aspartic acid--5.5%, glycine--4.6%, glutamic acid--8%, alanine--5% and phenylalanine--5.5%.

Methyl alcohol: In the quest for new, promising sources of carbon raw material for the microbiological industry, researchers are turning more and more to methyl alcohol. This is attributable to the fact that methanol has several advantages over other carbon-containing substrates [4-8]. Synthesis of unicellular protein from methanol may be more economical than the existing processes [9-10]. The expected drop in the price of methanol in the USSR may result in its being competitive with petroleum hydrocarbons [11].

Ye. I. Kvasnikov et al. have proposed a method of producing protein-vitamin concentrates from methanol with the yeast of the genus *Candida* [12]. Subsequently, as a result of long-term breeding, a strain of *C. boidinii* yeast was developed, the maximum growth rate of which is up to  $\mu$ -0.22  $h^{-1}$ , while its economic coefficient constitutes 33-35%.

The concentration of methyl alcohol in the medium and aeration conditions are important factors that affect the growth of methanol-utilizing yeast. A study of the effect of methanol concentration in the nutrient medium on growth of *C. boidinii* yeast in the course of a periodic process revealed that a maximum growth rate is observed with 0.2-1.0 vol.% methanol content. There is a decline in specific growth rate with methanol concentrations in excess of 1.5 vol.%. The range of methanol-limited growth rate is below 0.2 vol.% concentration, so that it is difficult to demonstrate the restrictive effect of methanol when a period cultivation process is used. For this reason,

the limiting concentration of methyl alcohol for growth of *C. boidinii* yeast was determined in the chemostat mode.

It was demonstrated, with regard to many microorganisms, that there is a correlation, expressed by the Michaelis-Menteb constant for enzymatic reactions, between the rate of microorganism growth under stationary conditions in a continuous process and minimum concentration of nutrient [13]. It is also known that usually more than one substrate is involved in enzymatic reactions, and any precise description of such reactions must take this fact into consideration [14].

In the case of chemostat cultivation of *C. boidinii*, a change in concentration of methanol in the nutrient medium with a constant dilution coefficient induces a change in methyl alcohol content in the cultural fluid [15]. This indicates that methanol is not the only limiting factor. Since there are excessive amounts of mineral components in the medium and of group B vitamins, the concentration of dissolved oxygen may be an additional grow-limiting factor. There is an inverse correlation between concentration of methanol and dissolved oxygen in cultural fluid in the case of chemostat cultivation. The same correlation is observed when there is a change in coefficient of oxygen mass transfer when the growth rate and methanol concentration in the nutrient medium are constant. Thus, the concentration of methanol and dissolved oxygen have a concurrent influence on the specific rate of growth of methanol-assimilating yeast.

In this case, the substrate reaction could be the "controlling" reaction, i.e., a reaction, the rate of which determines the growth rate of microorganisms.

Bisubstrate enzymatic reactions can be described by charts, in which there is formation of a triple or dual enzyme-substrate complex (ESC).

In charts [or schemes] that provide for formation of a triple ESC, there are four equiponderant reactions between an enzyme and oxidizer, enzyme and substrate, dual ESC (enzyme--oxidizer) and substrate, dual ESC (enzyme--substrate) and oxidizer.

The triple ESC dissociates with production of a product free of enzyme and reduced form of oxidizer. The following equation corresponds to this mechanism:

$$V = V_m \frac{S_1 \cdot S_2}{K_1 \cdot K_2 + K_1 \cdot S_2 + K_2 \cdot S_1 \cdot S_2},$$

the graphic rendition of which in coordinates  $\frac{1}{V} - \frac{1}{S_1}$  and  $\frac{1}{X} - \frac{1}{S_2}$  is in the form of straight lines that intersect one another above, under or on the x-axis.

For the scheme of a bisubstrate reaction involving formation of dual ESC, the oxidizer is the oxidized form of the coenzyme, which is continuously regenerated.

The rate of the reaction as function of substrate concentration is described by the following equation:

$$V = V_m \frac{S_1 \cdot S_2}{K_1 \cdot S_2 + K_2 \cdot S_1 + S_1 \cdot S_2},$$

the graphic rendition of which in coordinates  $\frac{1}{V} - \frac{1}{S_1}$  and  $\frac{1}{V} - \frac{1}{S_2}$  is in the form of parallel lines.

Data were obtained on the specific rate of growth of methanol-assimilating *C. boidinii* yeast as function of concentration of methanol and dissolved oxygen in coordinates  $\frac{1}{\mu} - \frac{1}{S}$  and  $\frac{1}{\mu} - \frac{1}{C}$ . The bisection of lines on the y-axis indicates that there is orderly formation of triple ESC, i.e., only one of the substrates forms the dual ESC.

In this case, the specific growth rate function is described by the following equation:

$$\mu = \mu_m \frac{S \cdot C}{K_{sc} + S \cdot C}.$$

Constants  $\mu_m$  and  $K_{sc}$  were determined for different strains of methanol-assimilating yeast by means of submitting experimental data in coordinates  $\frac{1}{\mu} - \frac{1}{S \cdot C}$ . The straight lines inherent in different strains bisect the y-axis at point  $\frac{1}{\mu_m}$ , and the x-axis at point  $\frac{1}{K_{sc}}$ . The maximum specific growth rate of three strains of yeast constitutes  $0.12-0.21 \text{ h}^{-1}$ . The saturation constant of methanol-oxidizing strains is virtually the same, and it constitutes 0.45-0.6 vol.% saturation. This is indicative of the existence of a common "controlling" reaction in the cultures studied.

The hypothesis that this "controlling" reaction may be the reaction catalyzed by oxidase (alcohol oxidase) was tested in the next experiments. Polarographically, using a membrane sensor of dissolved oxygen, determination was made of the rate of oxygen uptake by methanol-oxidizing *C. boidinii* yeast cultivated on a medium with glucose or methanol. The rate of methanol uptake by a culture raised on medium with glucose remains virtually constant until all the oxygen from the medium is consumed, and it diminishes with  $pO_2$  under 1% saturation. The rate of oxygen uptake by a culture raised on a medium with methanol begins to decline at higher partial oxygen pressure, and it depends on the concentration of methanol in the medium.

It was shown in several works that the rate of oxygen uptake diminishes with decline of partial pressure of dissolved oxygen below a specific critical concentration, which usually constitutes 0.1-3% saturation with air. The

studies of Chance et al. [16] revealed that, in this case, the rate of oxygen uptake is determined by the rate of the reaction catalyzed by cytochrome oxidase, which has a high affinity for oxygen.

Lozinov et al. (17) demonstrated a decrease in oxygen uptake by yeast raised on n-alkanes, with partial pressure of dissolved oxygen at 20-30% saturation, and they attribute this to the fact that the rate of oxygen uptake is determined by the rate of the oxygenase reaction, which is the first reaction of degradation of n-alkanes. The saturation constant for oxygenase oxygen is higher by a factor of  $10^1$ - $10^2$  than the constants for saturation of cytochrome oxidase, and for this reason the decline in rate of oxygen uptake begins at higher concentrations thereof.

It may be assumed that the rate of oxygen uptake by methanol-assimilating *C. boidinii* yeast raised on medium with glucose is determined by the rate of electron transfer at the cytochrome oxidase level; and in the case of oxygen uptake by cells raised on medium with methanol, the "controlling" reaction is not in the respiratory chain, and it is catalyzed by oxidase or oxygenase. Since the critical concentration of oxygen for yeast that grows on methanol changes as function of its concentration in the polarographic "mesh," most likely the enzyme of this reaction is alcohol oxidase, the first enzyme of oxidation of methyl alcohol.

Thus, we have demonstrated that the main kinetic function of growth of methanol-assimilating *C. boidinii* yeast is an equation with dual influence of concentrations of methanol and oxygen [15].

The stoichiometry of growth of methane-oxidizing *C. boidinii* yeast was studied by means of a balance of macroergic bonds [18]. On this basis, equations were developed for economic coefficients for methanol and oxygen as function of cultivation conditions. The experiments revealed that these indices decline with increase in concentration in the medium. Intensification of free oxidation is the cause of this phenomenon.

The balance of ATP for *C. boidinii* raised on medium with methanol has the following appearance:

$$\frac{1}{Y_{kat}} \cdot 2P/O = \frac{1}{3} \cdot \frac{1}{Y_{acc}} \cdot \frac{1}{Y_{ATP}}$$

In the left part of the equation is shown ATP formation with oxidation of methanol and in the right, ATP consumption for assimilation of methanol to phosphoglycerin aldehyde and ATP consumption for synthesis of biomass from triosephosphate.

As a result of investigating the physiology of methanol-assimilating yeast, a mathematical model of the cultivation process was developed, and it has the following appearance:

$$\mu = D = \mu_m \frac{S \cdot pO_2}{K_{s,pO_2} + S \cdot pO_2} \quad (1)$$

$$D_x = Y_{o_2} \cdot K_v \cdot C_H \cdot 10^{-2} (100 - pO_2) \quad (2)$$

$$X = Y_s (S_0 - S) \quad (3)$$

$$Y_s = \frac{pO_2^2 + 1800}{3,5pO_2^2 + 4350} \quad (4)$$

$$Y_{o_2} = \frac{pO_2^2 + 1800}{6,65pO_2 + 3600} \quad (5)$$

where  $\mu$  is the specific growth rate,  $D$  is the dilution coefficient,  $S_0$  and  $S$  are concentration of methanol in the nutrient medium and cultural fluid,  $pO_2$  is partial pressure of dissolved oxygen,  $C_H$  is saturating oxygen concentration,  $K_v$  is the volumetric coefficient of mass transfer,  $Y_0$  and  $Y_s$  are the economic coefficients for oxygen and methanol. These equations correspond to the established states of chemostat cultivation of *C. boidinii* on a medium with methyl alcohol.

Equation (1) reflects the phenomenon of dual influence of concentrations of oxygen and methanol; equations (2) and (3) refer to the tangible balance of methanol and oxygen. Equations (4) and (5), which describe the economic coefficients as function of  $pO_2$ , were obtained from an analysis of patterns of free oxidation in methanol-assimilating yeast.

A computer was used to calculate the system of equations with values of  $K_v$  of 100, 500 and 1000  $h^{-1}$ , concentrations of methanol in cultural fluid of  $S_0 = 1, 2, 4, 6, 7, 10$  and 12 vol.% and coefficients of dilution  $D = 0.04, 0.06, 0.08, 0.12, 0.14, 0.16, 0.18, 0.20$  and  $0.20 h^{-1}$ .

Experimental industrial cultivation of *C. boidinii* yeast was performed on the Drogobychskaya unit for protein and vitamin concentrates. The inoculum was cultivated successively in fermenters with a working capacity of 100  $l$ , 400  $l$  and 2  $m^3$ . The main cultivation was performed in a fermenter with a working volume of 7-8  $m^3$ .

The tests were performed with a dilution coefficient of 0.10 and  $0.13 h^{-1}$ . The concentration of biomass in the fermenters was maintained at the level of 3.5-4.0 g ASD [expansion unknown]. The yield of biomass according to provided methanol constitutes 31-36% and according to assimilated methanol, 40%. The yeast biomass was characterized by a high crude protein content, 57-60%, and low lipid content, 0.1-0.2%.

The results of laboratory studies and experimental industrial tests served as the basis for development of technological regulations for the production of feed yeast based on methyl alcohol.

A study has been made of the process of production of feed yeast from agricultural by-products--rice and wheat straw, sunflower stalks, grapevine cuttings, spent tanbark, flax and hemp fiber--the resources of which constitute over 15 million tons in the Ukrainian SSR. Production tests of the process have been performed.

Studies have been conducted of the physiology of methanol-assimilating yeast, and a mathematical model of the process of cultivation thereof was prepared. Optimum cultivation conditions have been determined on the basis of estimations made on the mathematical model.

Laboratory studies and industrial tests are indicative of the feasibility of industrial production of feed yeast based on methanol.

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## INDUSTRIAL MICROBIOLOGY

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### WOOD CHEMISTRY HYDROLYSIS INDUSTRY PROGRESS

Moscow GIDROLIZNAYA I LESOKHIMICHESKAYA PROMYSHLENNOST' in Russian No 3, 1978 pp 1-2

[Article by I.P. Doshlygin, Main Administration for the Microbiological Industry of the USSR Council of Ministers: "Work Better Today Than Yesterday, and Tomorrow Better Than Today!"]

[Text] In 1977 the hydrolysis industry increased the product sales volume by 7.7 percent as compared with 1976.

The production of nutrient yeasts rose by 6.9 percent, of furfural--by 5.6, of premixes based on nutrient yeasts--by 33.8 and of xylite--by 8.9 percent. The profit from the production work increased by 79 percent.

An improvement was achieved in a number of qualitative indicators. For example, the output of sugars made of refined raw material was 41.1, as compared with 40.0 percent in 1976, the output of nutrient yeasts made of sugars increased by 4.6 percent, and of alcohol made from raw material--by 3 percent.

In competing for economical consumption of raw material and an increase in product output, the collectives of the Volzhskiy Yeast Hydrolysis, the Kirovskiy Biochemical and the Tavda Hydrolysis plants achieved an output of nutrient yeasts from each ton of wood raw material of 200 kilograms and more. The collectives of the Bobruysk, Zima, Kedaynyay and Kansk plants came very close to this milestone. The raw protein content in the yeasts on the average was 52.8 percent.

The Astrakhan', Volgograd, Arkhangel'sk, Onega and other plants produced yeasts with a high protein content, reaching 53-55 percent.

In 1977, in the production of nutrient yeasts, as compared with the norms, on the whole for the hydrolysis industry there was a saving of: 74,000 cubic meters of wood raw material, about 3000 tons of sulfuric acid and over 11,000 tons of nutrient salts. The saving in thermal energy was

321,000 gigacalories and in electric energy--44.8 million kilowatt-hours. An economic saving of 4.9 million rubles was obtained from introducing inventions and efficiency expert proposals during the year.

Four types of products were certified additionally last year for the State Seal of Quality.

The highest results in fulfilling the plan for 1977 and the socialist commitments adopted were achieved by the collectives of the Kirovskiy Biochemical Plant, the Andizhan, Arkhangel'sk, Bobruysk, Ivdel' and Khor Hydrolysis plants and the Zaporozh'ye Yeast Hydrolysis Plant. For example, the collective of the Kirovskiy Biochemical Plant, having started a special shock work effort in 1976 in honor of the 60th anniversary of the Great October Revolution, overfulfilled the assignments for two years of the five-year plan for the production and sale of industrial products by 3.6 million rubles.

For achieving the highest results in socialist competition to increase production efficiency and raise work quality and ensure stable indicators in fulfilling the national economic plan and increased commitments for 1977, the collective of the Kirovskiy plant was awarded the Red Challenge Banner of the CPSU Central Committee, the USSR Council of Ministers, the All-Union Central Trade Union Council and the Central Committee of the All-Union Lenin Young Communist League.

Recognized as winners in the All-Union Socialist Competition in 1977 and awarded Red Challenge Banners of the Main Administration of the Microbiological Industry at the USSR Council of Ministers and the Central Committee of the Trade Union of Workers in the Chemical and Petrochemical Industry, with the investiture of the first monetary prizes were the collectives of the Bobruysk and Khor plants, the second monetary prizes--of the Andizhan and Ivdel' plants and of third prizes--of Arkhangel'sk and Zaporozh'ye plants. The good work of the Krasnoyarsk Biochemical Plant was noted.

Recognized as winners of the All-Union Socialist Competition among the Komsomol youth brigades (shifts) of the microbiological industry were the brigades of the ethyl alcohol shops of the Bobruysk and Tavda Hydrolysis plants, headed by T.A. Maz'ko and M.M. Plekhov.

In the competition of the brewers' brigades of the hydrolysis shops the brigades of T.N. Zalesova (Ivdel' plant) and V.V. Shishkin (Arkhangel'sk plant) were the winners.

The highest results in the competition of the brigades (shifts) for the production of nutrient yeasts were achieved by the shift of Yu.A. Starikova (Tavda plant), and the brigades of L.P. Khlopko (Azporozh'ye plant), V.G. Bulanova (Khor plant) and V.A. Kolupayev (Kirovskiy plant).

Right-flank members in the competition for the yeast cultivation operators are: T.S. Fominykh, hero of Socialist Labor (Tavda plant), V.V. Osipova (Kirovskiy plant), A.M. Mingaleva (Lobva plant) and M.I. Tsekhanovich (Bobruysk plant).

The winners in the competition by occupations were: brewers Yu.A. Guritskiy (Zaporozh'ye plant), S.S. Astapov (Bobruysk plant), V.M. Vilisov (Ivdel' plant); instrument control operators M.Z. Novoselova (Khor plant), S.A. Golikov (Andizhan plant); dryer operator A.M. Korkin (Arkhangel'sk plant); separator A.P. Vyatkina (Krasnoyarsk plant); equipment repair man A.M. Karepov (Manturovo plant) and V.M. Smolenchuk (Rechitsa plant).

In the third year of the five-year plan the workers in the hydrolysis industry are to increase substantially the production volume, raise its efficiency and product quality.

The state plan for 1978 specifies a 6.2 percent product sale increase as compared with 1977, a 7.0 percent increase in the production of commodities to be marketed and a 2.1-fold increase in the output of the highest quality category.

The output of basic types of products will increase: of nutrient yeasts--by 6.3 percent, of ethyl alcohol--by 1.7, of premixes--by 25.6, of furfural--by 3.0 and of xylite--by 34.6 percent.

Labor productivity must increase by 5.7 percent, and over 80 percent of the increase in products to be marketed must be obtained by means of this; the profit from the industrial work is to increase by 50.3 percent.

To fulfill the tasks set all the intraproduction reserves must be mobilized, socialist competition widely developed, its organization improved and the effectiveness increased.

There should be radical intensification of work on studying and disseminating advanced production experience.

A specific program of actions for all the workers in industry, helping to concentrate the efforts on the key tasks, was set forth in the Letter of the CPSU Central Committee, the USSR Council of Ministers, the All-Union Central Trade Union Council and the Central Committee of the All-Union Lenin Young Communists League "On Developing Socialist Competition for the Fulfillment and Overfulfillment of the Plan for 1978 and Intensifying the Struggle to Raise Production Efficiency and Work Quality." In response to the resolutions of the CPSU Central Committee December (1977) Plenum, the speech made at it by Comrade L.I. Brezhnev and in response to the Letter of the CPSU Central Committee the USSR Council of Ministers, the All-Union Central Trade Union Council and the Central Committee of the All-Union Lenin Young Communists League, the collectives of the Kirovskiy Biochemical Plant, 34 shops and about 1200 workers of the hydrolysis enterprises pledged themselves

to fulfill the assignments of the three years of the Tenth Five-Year Plan by 7 October 1978--USSR Constitution Day. The collectives of the Bobruysk Hydrolysis Plant and many shops, sections and shifts of other enterprises resolved to complete the fulfillment of the assignments of three years of the five-year plan by the 61st anniversary of the Great October Revolution. All the collectives of the enterprises adopted commitments to fulfill and overfulfill the State Plan for 1978. Many enterprises adopted counter plans.

The initiative of the Kirovskiy biochemists on developing socialist competition for utmost increase in production and the honored right to participate in producing the millionth ton of nutrient yeasts since the beginning of the current year was taken up and broadly disseminated.

The collectives of the Bobruysk, Tavda, Khakasskiy, Volgograd and Zaporozh'ye plants and the Dal'vostokgidrolizprom, Irkutskgidrolizprom and the Moldavgidrolizprom production associations and the Gidrolizprom Scientific Production Association are working successfully this year. They have achieved high growth rates in production volumes and labor productivity.

As was noted at the meeting of the board of Glavmikrobioprom [Main Administration for the Microbiological Industry], however, a number of plants of the hydrolysis industry, having entered in 1978, failed to secure the progress achieved. Failures in the work of the Severnyy, Ural and Siberian plants, caused by delays in the supplies of raw material and fuel, appeared by the beginning of January.

The Board of Glavmikrobioprom noted that the late preparation for the fall-winter period should be considered one of the reasons for the unsatisfactory work of a number of hydrolysis plants in the first quarter of 1978. In particular, certain problems of material-technical supply were not solved on schedule and insufficient attention was paid to organizing production and its administration.

The Soyuzgidrolizprom All-Union Industrial Association acted correctly, concentrating its attention on the work of plants formerly lagging behind and new plants, developing capacities. As a result, it succeeded in getting out of trouble the Khakasskiy Hydrolysis and the Kedaynyay and Lesozavodskiy Biochemical plants, the Moldavgidrolizprom Production Association and certain other enterprises. The Khakasskiy Hydrolysis Plant, for example, in January 1978 was working with a smooth flow and achieved an average daily output of nutrient yeast of over 80 tons. The volume of production and product sales at this enterprise rose by 61 percent as compared with January 1977 and the labor productivity rose 1.5-fold.

Many other plants have also succeeded in achieving an efficient, smooth flow of work since the first days of the year. In being occupied with the plants that were lagging behind, however, Soyuzgidrolizprom lost sight of the plants that had formerly been considered satisfactory. Some of them did not fulfill the plan for the first quarter of 1978.

The hydrolysis industry is faced with setting up strict supervision over adherence to plan discipline, specific norms for materials and particularly power input for the output of a unit of the product, the deadlines for the arrival of raw material and supplies of the finished products to the consumers.

Further development of socialist competition is important for fulfillment of the year's plan by the hydrolysis industry.

In October 1977 Glavmikrobioprom and the central committee of the trade union approved the conditions for the All-Union Socialist Competition of the collectives of the associations and enterprises, scientific research and planning organizations, brigades, shifts and workers in the key occupations to increase production efficiency and raise work quality and for successful fulfillment of the assignments of the Tenth Five-Year Plan.

In his speech at the CPSU Central Committee December (1977) Plenum, Comrade L.I. Brezhnev once again emphasized the main tasks of the production workers: "Work better today than yesterday, and tomorrow--better than today. This is the motto of the day. And better--means stress on quality, on efficiency and on a rise in labor productivity."

The new conditions for socialist competition are also directed precisely toward mobilizing the work collectives and all the sector's workers to the utmost rise in labor productivity, increase in the proportion of products of the highest quality in the total production volume, fulfillment of the plan for production and supplies of goods on the product list and by the deadlines specified by the orders and economic agreements, fuller utilization of the production capacities and an increase in the output-capital ratio, a reduction in expenditures to produce the goods and an increase in profit.

Among the chief indicators of the competition are putting into operation fixed capital, production capacities, apartment houses and facilities for cultural and everyday purposes, as well as the state of the labor conditions and labor safety techniques at the production facility.

In summing up the results of the competition, many other indicators of the collectives' production work are analyzed and taken into consideration, in particular, the course of fulfilling the counter plan and commitments taken on, the level of organization of intraplant socialist competition, the state of the inventiveness and efficiency expert work, the level of mechanization of labor-intensive and heavy work, norm-setting for labor, the introduction of scientific organization of labor and the Shchekino method, the output of goods made of processed raw material and the content of active substance in goods produced, fulfillment of the plan for social development of the collective and the comprehensive plan for improving work conditions, sanitation and health measures and nature conservation, the state of the personnel turnover, labor discipline, adherence to the norms for social order, etc.

The collective's work is evaluated in consideration of the results not only for the period under review, but also since the beginning of the year and of the five-year plan.

The conditions for the All-Union Socialist Competition of scientific research organizations reflect the special features of the work of their collectives and are aimed at increasing the efficiency of scientific research, achieving a high scientific-technical level and quality of studies, and acceleration of their being introduced into production, obtaining the appropriate economic effect. The collectives of the planning organizations are oriented toward providing the facilities being constructed and renovated, on schedule, with technical documentation worked out with the use of the newest achievements of science, technology and advanced experience.

The brigades (shifts) and workers in the key occupations compete to achieve certain milestones. For example, the brigades of brewers of the hydrolysis shops struggle for the highest extraction of sugars, on the average for the year, from each cubic meter of capacity of the hydrolysis equipment and from each brew, with a saving of raw material and steam, as compared with the specific consumption norms; the brigades (shifts) of the yeast shops--for maximum output of commercial yeasts made of processed raw material and the largest extraction of a marketable product of high quality with the equipment installed.

A saving of energy resources occupies an important place under the conditions of socialist competition.

It is extremely important in all the work collectives to carry out at a high level the All-Union Public Inspection of the efficiency of using raw material, materials and fuel and energy resources. The Letter of the CPSU Central Committee, the USSR Council of Ministers, the All-Union Central Trade Union Council and the Central Committee of the All-Union Lenin Young Communists Youth League said that to save on everything, both large and small, to reduce production cost and to overcome completely cases of squandering and wastefulness is the integral requirement of socialist economic operations and the duty of all the workers.

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MORPHOLOGICAL CHANGES IN THE MUCOUS MEMBRANE OF RESPIRATORY TRACTS UNDER  
EFFECT OF METAL COBALT AEROSOL IN CHRONIC EXPERIMENT

Kiev ZHURNAL USHNYKH NOSOVYKH I GORLOVYKH BOLEZNEY in Russian No 3, 1978  
pp 41-45

/Article by G. A. Georgiadi and L. A. El'kind, from the North Ossetian State  
Medical Institute (Prof A. Kh. Adyrkhayev, rector) and the Tashkent Medical  
Institute (Prof U. A. Aripov, academician of the Uzbek SSR Academy of Sci-  
ences, rector)/

/Text/ Under the present conditions of hydrometallurgical cobalt production the entry of metal cobalt aerosol into the atmosphere of production premises is possible at some sections (T. M. Urusova, 1967). An excess entry of cobalt into the human body produces marked deviations in the state of health (Z. S. Kaplun, 1955; G. A. Georgiadi and A. S. Ioanesyan, 1969 et al.). Acute respiratory diseases, chronic bronchitis, catarrhal inflammation of the mucous membrane of the entire respiratory tract, dermatitis and fibrous changes in the lungs are often noted in workers subjected to the effect of metal cobalt aerosol (T. A. Kochetkova, 1960; F. I. Kolpakov and A. F. Kolpakova, 1968; Z. Ye. Dzukayev, 1972 et al.).

Numerous experimental investigations confirmed the toxic effect of cobalt and its compounds on the internal organs of animals with various ways of administration (G. M. Parkhomenko, 1948; Ye. V. Khukhrina, 1956; E. N. Levina and N. A. Minkina, 1961; Z. S. Kaplun, 1963; O. N. Gurtsiyev, 1967 et al.). At the same time, the effect of cobalt on the mucous membrane of respiratory tracts as the boundary surface in contact with the toxic agent remains unclarified.

Examining workers subjected to the effect of cobalt aerosol and its compounds, we noted dystrophic and atrophic changes in the mucous membrane of their respiratory tracts (G. A. Georgiadi, 1976). A significant number of bronchitides were also detected in this category of workers. This impelled us to conduct investigations on animals for the purpose of clarifying the morphological changes in the mucous membrane of the respiratory tract under the effect of the dust of the indicated metal.

The experiment was conducted in the Laboratory of Toxicology (Prof I. V. Sanotskiy, head) of the Scientific Research Institute of Labor Hygiene and Occupational Diseases of the USSR Academy of Medical Sciences on 57 white rats of both sexes. When the rats were selected, the weight of an animal was primarily taken into consideration as the most integral indicator. Chronix exposure was carried out for 4 months, after which the animals were under observation for 30 days. A uniform distribution of metal cobalt dust in the chamber was ensured by an electric ventilator. Cobalt concentration was  $0.48 \text{ mg/m}^3$ , which corresponded to the existing maximum permissible concentration. Dust dispersion was no more than  $5 \mu$ . Exposure was carried out 4 hours a day 5 times a week for 4 months. The control group, which consisted of 30 rats, was under similar conditions, but was not subjected to the effect of metal cobalt dust.

The animals were killed by decapitation 2, 4 and 5 months after the beginning of exposure. The nasal septum with the mucous membrane, larynx, trachea and lungs were fixed in neutral formalin and immersed in paraffin. The prepared sections were stained with hematoxylin-eosin.

Pronounced circulatory disorders in the form of swelling of the cells of the covering epithelium, intercellular edema, pronounced edema of the connective tissue of the mucous membrane of the nose and of the tela submucosa of the larynx, trachea and bronchi, dilatation and hyperemia of vessels and massive and, here and there, diffuse lymphohistiocytic infiltrates in the mucous membrane of respiratory tracts were seen in it after 2 months (fig. 1, a).

At the same time, dystrophic changes in glandular and covering epithelium cells were observed in individual sections of the mucous membrane, especially in the nasal cavity. These changes were expressed in albuminoid degeneration. Desquamation of the epithelium in the lumen was noted in the mucous membrane of the nasal cavity and, here and there, in the mucous membrane of the bronchi (fig. 1, 6).

Comparing the afflictions of the mucous membrane of respiratory tracts in the males and females, it could be noted that all the pathomorphological processes were more pronounced in the males.

After 4 months, along with pronounced circulatory disorders in the mucous membrane of respiratory tracts on the part of the covering epithelium, albuminoid (mucous and dropsical) degeneration was observed. An increase in the number of goblet cells in the mucous membrane of the respiratory zone of the nasal cavity, as well as large sections of gelatinization of the covering epithelium also extending to the olfactory region, was noted (fig. 1, b). Furthermore, exfoliation of part of the epithelial layer and desquamation of epithelial cells were noted in almost all the sections of respiratory tracts. Pronounced edema, here and there homogenization of collagenous fibers and loosening and intumescence of vascular walls, sometimes with phenomena of plasmorrhagia, were noted in the mucous membrane of the nasal cavity and the larynx and in the tela submucosa of the trachea and bronchi. In addition to

this, diffuse and massive lymphoplasmohistiocytic infiltrates permeating the mucous membrane and the tela submucosa and here and there penetrating into the epithelial layer were observed (fig. 1, г).

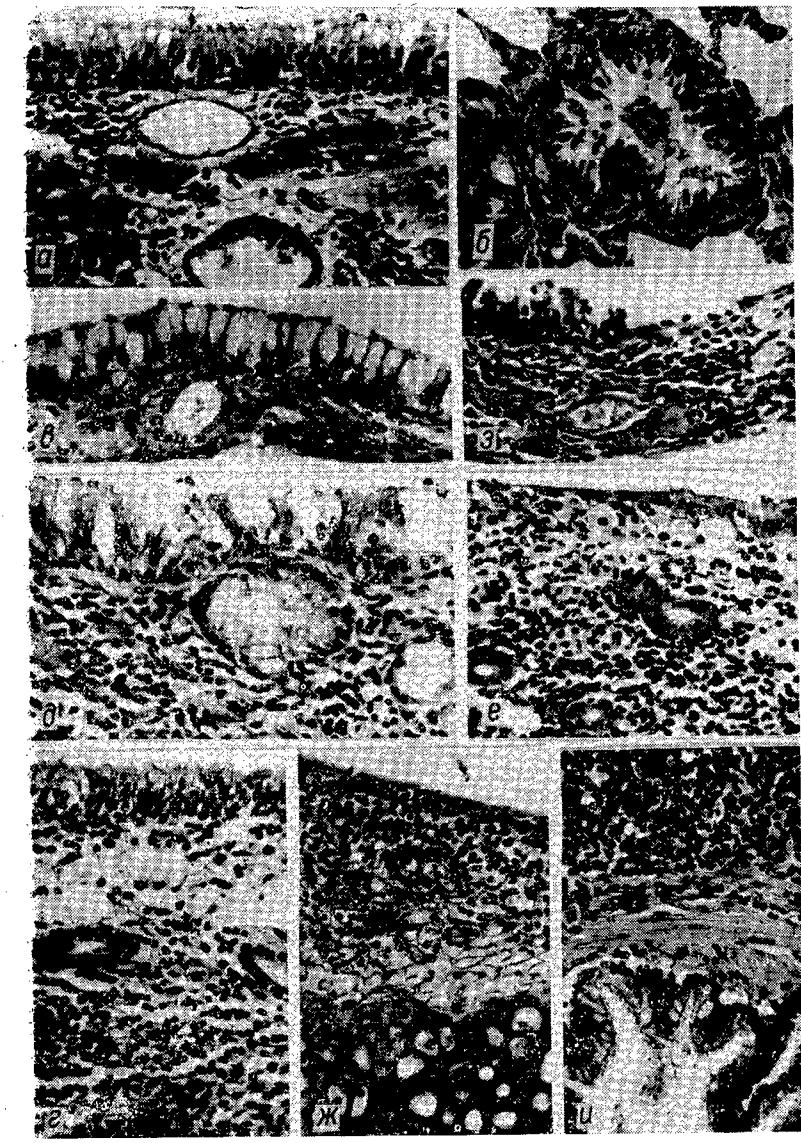


Fig. Pathomorphological changes in various sections of the mucous membrane of respiratory tracts in rats under an inhalation effect of metal cobalt dust: а, в, г, д, з, е--the mucous membrane of the nasal cavity; ж--the mucous membrane of the trachea; б и--of the bronchi. Staining with hematoxylin-eosin. Enlargement 10X20.

On the part of the glandular cells of mixed glands and glands of the olfactory region swelling, vacuolization, pronounced albuminoid degeneration, as well as necrobiosis and necrosis phenomena (homogenization of the cytoplasm,

wrinkling and condensation of the nucleus and absence of staining), occurred. The pathological process also involved the cartilaginous tissue, where a swelling of cartilaginous cells and a change in the tinctorial properties of the intercellular substance were noted.

The changes of a circulatory and dystrophic nature enumerated above were more intensively pronounced in the males than in the females.

One month after the cessation of exposure restorative processes were not observed on the part of the mucous membrane of respiratory tracts. Pronounced circulatory disorders in the form of dilatation and hyperemia of blood vessels and marked dystrophic changes in the covering epithelium and in the glandular cells of mixed glands remained.

Large sections of gelatinization of the covering epithelium and individual spots where epithelial cells were in a state of destruction remained in the mucous membrane of the nasal cavity of both the respiratory and olfactory region (fig. 1, *д*). This attests to the depth of affection of the mucous membrane, insufficiency of its repairability and disturbance in cellular metabolism.

In the mucous membrane of all the sections of air-bearing tracts, along with pronounced dystrophic changes in the form of albuminoid degeneration, atrophy of the epithelial layer represented by a layer of flattened cells was detected here and there (fig. 1, *е, ж*). Sections where the epithelial layer was absent and the lining connective tissue was denuded occurred (fig. 1, *з*). Individual sections of metaplasia, where instead of columnar ciliate epithelium stratified squamous epithelium occurred were visible in the mucous membrane of the larynx and trachea. Pronounced edema remained in the connective tissue of the mucous membrane and the tela submucosa. Thickening of bronchial and vascular walls as a result of the proliferation of the connective tissue was noted. Massive focal and diffuse lymphohistiocytic infiltrates were seen around the bronchi (fig. 1, *и*).

In the males more extensive sections of the covering epithelium were subjected to atrophy. More massive round-celled infiltrates were noted.

#### Conclusions

An inhalation effect of metal cobalt aerosol at the level of the existing maximum permissible concentration leads to the development of circulatory, dystrophic and, here and there, atrophic changes in the mucous membrane of respiratory tracts.

One month after the cessation of exposure normalization of the morphological structure of the mucous membrane of the respiratory tract is not observed, which is also confirmed by our histochemical investigations (G. A. Georgiadi, 1978).

Apparently, the detected characteristics of the change in the mucous membrane of respiratory tracts in the males and females are connected with sex differences in this intoxication.

Thus, a chronic inhalation exposure to metal cobalt dust in a concentration of  $0.48 \text{ mg/m}^3$  leads to irreversible changes (atrophy) in the mucous membrane of the respiratory tract of experimental animals.

It can be assumed that dystrophic and atrophic changes in the mucous membrane of respiratory tracts in metal ceramics enterprise workers are connected with a prolonged effect of metal cobalt aerosol.

The pathomorphological data obtained, along with the histochemical changes in the mucous membrane of the respiratory tract of experimental animals and clinical observations, which we described earlier, can be the substantiation for a revision of the existing maximum permissible concentration of metal cobalt dust in the atmosphere of a work zone.

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HISTOMORPHOLOGICAL CHANGES IN THE ACOUSTIC ORGANS OF EXPERIMENTAL ANIMALS  
UNDER PROLONGED EFFECT OF INDUSTRIAL NOISE AND VIBRATION

Kiev ZHURNAL USHNYKH NOSOVYKH I GORLOVYKH BOLEZNEY in Russian No 3, 1978  
pp 60-65

Article by Prof Yu. M. Guzeyev, G. T. Shkromida, and Candidate of Medical Sciences N. P. Zbirak, from the Department of Otorhinolaryngology (Prof Yu. M. Guzeyev, head) and the Department of Patho-anatomy (Prof N. A. Sheremeta, head) of the Ivano-Frankovsk Medical Institute (Prof G. A. Babenko, UkrSSR honored scientist, rector)

Text As a result of repeated examinations of workers at compressor shops of a gas processing plant symptoms of neuritis in acoustic nerves were diagnosed in 44.02% (Yu. M. Guzeyev, G. T. Shkromida, M. I. Shkromida, L. A. Shvedenko and R. D. Gerasimchuk, 1974). Sanitary and hygienic investigations established that noise at the plant shops exceeded the permissible norms by 2 to 19 db, and vibration, by 6 db, which under a prolonged and combined effect on the human acoustic organ can lead to the development of an occupational hearing disorder (Ye. P. Andreyeva-Galanina, S. V. Alekseyev, and A. V. Kadyskin, 1970; L. A. Kirsanova, 1972; A. Ya. Yurkevich and M. L. Khaymovich, 1974; T. A. Tret'yakova, 1963, 1974; Vaffe, Jones and Weissing, 1958; Jorgensen, 1963).

In the literature there are indications on the effect of noise and vibration on the bodies of experimental animals (N. F. Popov, 1927; B. S. Preobrazhenskiy, 1929; S. S. Grobshteyn and A. V. Kugaro, 1931; V. N. Yerokhin, 1968; A. A. Korniyenko, 1972; Falk et al., 1974; Chodynicki and Hermanowicz, 1976). As a rule, however, these investigations were conducted under laboratory conditions. There are only isolated reports on the effect of industrial noise and vibration on the acoustic organs of experimental animals (T. A. Tret'yakova and A. K. Pokotilenko, 1976).

For the purpose of studying the morphological changes in the acoustic organ under a combined effect of noise and vibration cages with experimental animals were placed at a compressor shop at a distance of 1.5 m from compressor installations, that is, where individuals servicing these units spend most of their work time. A total of 12 rabbits of the chinchilla breed weighing 2.5

to 3.0 kg were used in the experiment. The experiment lasted 15, 30 and 60 days. The animals were kept on the usual ration. There was no death among the animals during the experiment. Upon conclusion of individual periods of observation the animals were killed. Pieces of tissues were taken from their internal organs. They were fixed in neutral formalin and in Carnoy's fluid and condensed in paraffin and celloidin. Then serial sections were prepared and stained with hematoxylin-eosin, and for glycogen, according to Shabadash with fermentative control with amylase.

Taking the characteristics of morphological investigations into consideration, the skull was opened and the material was taken from the temporal bone for a study of the acoustic organ in the following way. In six animals 1 day before the opening of the skull a 10%-formalin solution was perfused through the internal carotid artery under pressure. In the other six rabbits, after the removal of their heads, soft tissues were removed mechanically, the skull was cut in the sagittal direction and immersed in a 10%-neutral formalin solution and after 1 day the brain was separated, without disturbing the dura mater, in the region of the pyramid of the temporal bone. All these measures were taken in order to avoid mechanical injuries to the eighth pair of the cranial nerves. The disjointed temporal bones were decalcified for 7 days, after which part of the trochlea was immersed in celloidin, and part, in paraffin. Serial sections 7 to 10  $\mu$  thick were made from the preparations and subsequently studied under the light microscope.

The study of histopreparations of the acoustic organ in animals subjected to a sound and vibration effect under conditions of a compressor shop for 15 days showed the presence of disorders in circulation in all its sections: plethora, edema and often perivascular hemorrhage. Destructive changes were not observed.

We noted more pronounced shifts in the structures of the acoustic organs in three animals subjected to the effect of noise and vibration for 30 days. Marked disorders in circulation, that is, plethora, edema and perivascular hemorrhage, were detected in all the sections of the acoustic apparatus. At the same time, during the development of exudative inflammation dystrophic and desquamative changes in the epithelium of the tympanic cavity were observed. The tympanic membrane was loosened. Desquamation of the covering epithelium and swelling of the collagen fibers of the median layer were noted on the internal surface of this membrane. The vessels of the mucous membrane of the tympanic cavity were dilated sharply. The subepithelial layer was loosened and impregnated with a protein mass. Focal hemorrhage was around the vessels. Serous exudate with a mixture of isolated erythrocytes accumulated in the tympanic cavity (fig. 1, a). More pronounced changes were established in the labyrinth. At the same time, the most severe disturbances were detected in the nerve elements of Corti's organ. The contours of hair and sustentacular cells were indistinct, their nuclei were pyknotic and the cytoplasm was swollen. The covering membrane was shortened (fig. 1, 6). Disintegration of individual cells, their swelling with an eccentric disposition of nuclei and pyknosis were noted in the spiral ganglion. Cells with phenomena of vacuolar dystrophy and focal proliferation of glial elements were detected (fig. 1, B). Along with this the ganglion vessels were dilated sharply and their walls were loosened.

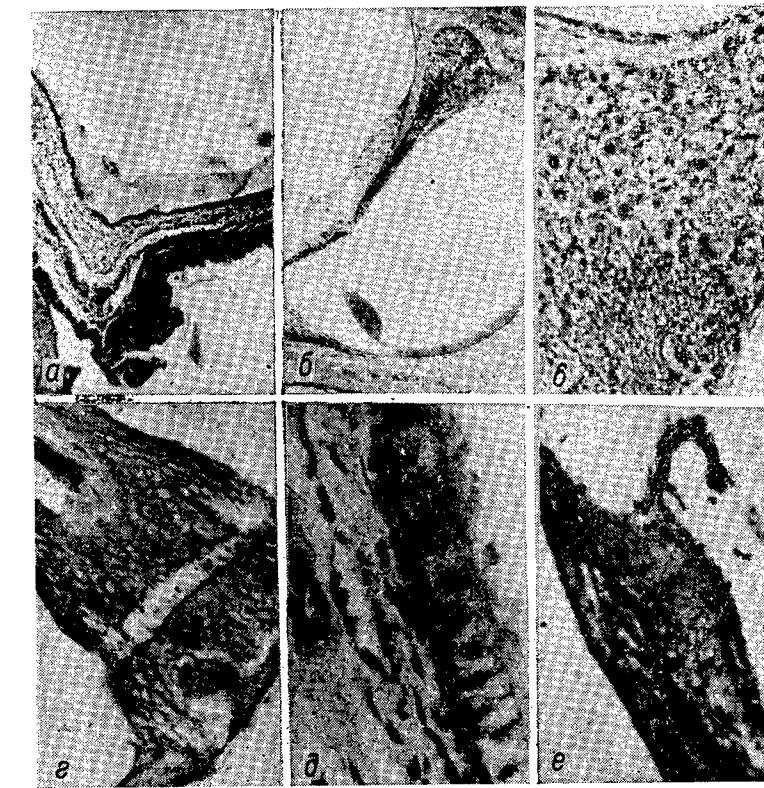


Fig. 1: a) desquamation of the epithelium and accumulation of serous exudate in the tympanic cavity. Hematoxylin-eosin. Ob. 8. Oc. 10; b) dystrophic changes in Corti's organ. Accumulation of serofibrinous exudate in the cochlea lumen. Hematoxylin-eosin. Ob. 8. Oc. 10; c) dystrophic changes in the cells of the spiral ganglion (edema and indistinct contours). Hematoxylin-eosin. Ob. 20. Oc. 10; d) resorption of the bone tissue of auditory ossicles with replacement of the foci of resorption by the connective tissue. Hematoxylin-eosin. Ob. 40. Oc. 10; e) dystrophic changes in the sensory cells of the crests of labyrinths. Hematoxylin-eosin. Ob. 40. Oc. 10; f) atrophy of the cells of Corti's organ. Hematoxylin-eosin. Ob. 40. Oc. 10.

Thus, by the 30th day of the experiment sharp disorders in circulation accompanied by an accumulation of serous exudate in the cochlea lumen and by dystrophic changes in the hair and sustentacular cells of Corti's organ were noted in all the sections of the acoustic organ.

The study of the changes occurring in the acoustic organ on the 60th day of the experiment showed that in all the structures of the middle ear there were certain disturbances expressed in dystrophic and inflammatory changes manifested in the plethora of vessels, resorption of the bone tissue and accumulation of serous fluid in the tympanic cavity with a negligible admixture of

leukocytes. The fibrous basis of the tympanic membrane was thickened, its capillaries were dilated sharply and the covering epithelium was swollen with desquamation sections. We also detected similar dystrophic changes in the epithelium lining the walls of the tympanic cavity. Edema of the lining connective tissue with loosening of collagenous fibers, dilatation of blood vessels and perivascular histiocytic infiltration were noted there. Vascular walls were loosened and an accumulation of the protein mass with an admixture of formed blood elements, primarily leukocytes, was noted in their lumen. Fatty degeneration phenomena were observed in the muscles of the tympanic cavity and the tendinous filaments at the places of their attachment were homogenized and partially fragmented. Auditory ossicles were also subjected to changes. Resorption sections with growth of the dense connective tissue were noted in their thickness, especially near joint connections (fig. 1, г).

Vascular plethora and dystrophic changes in the cells of Corti's organ and the spiral ganglion were also noted in the inner ear. The basal membrane of epithelium lining the cochlea was loosened, the epithelium was often cast off and an accumulation of calcium salts under it was noted. Significant changes were also observed in the cellular structures of the ampullary crests of semicircular canals. The contours of cells were slightly discernible, their nuclei were reduced and receptor cells were not determined (fig. 1, д). Loosening of the subepithelial layer of semicircular canals and desquamation of the covering epithelium occurred. It should be noted that destructive changes were more pronounced in the hair and sustentacular cells of Corti's organ. The cell cytoplasm was granular and the nuclei were pyknotic. The covering membrane was shortened and moved up (fig. 1, е). Vascular plethora, edema and loosening of the vascular wall with perivascular lymphoid-cellular infiltration were observed in the vascular stripe of the cochlea (fig. 2, а). Loosening and foci of desquamation of the epithelial cover of Reissner's membrane were noted (fig. 2, б). Vacuolization of the cytoplasm and pyknosis of the nuclei with the corrugation of neurons and a microglial reaction were seen in the spiral ganglion (fig. 2, в) and proliferation of glial cells along the nerve trunk was noted (fig. 2, г). Dystrophic changes and hemodynamic shifts (fig. 2, д) were also detected along the acoustic nerve located outside the temporal bone.

Sections of the temporal lobe of the brain corresponding to the cortical section of the acoustic analyzer were taken for an examination of the changes in the brain. At the same time, dystrophic changes in nerve cells with a microglial reaction were detected (fig. 2, е).

Under the production conditions studied by us, in addition to noise as the basic production factor, mechanical shaking-vibration is also important. Therefore, a combined effect of noise and vibration took place in our investigation.

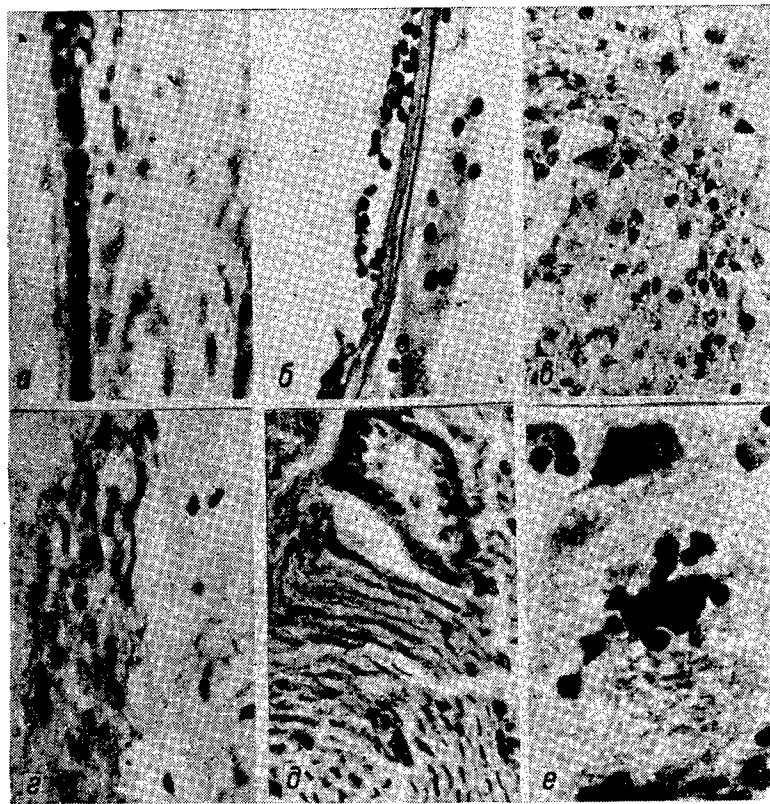


Fig. 2: a) productive inflammatory reaction in the vascular stripe of the labyrinth. Hematoxylin-eosin. Ob. 40. Oc. 10; б) edema and desquamation of the epithelium of Reissner's membrane. Hematoxylin-eosin. Ob. 40. Oc. 10; в) dystrophic changes in the cells of the spiral ganglion. Hematoxylin-eosin. Ob. 40. Oc. 10; г) proliferation of glial cells along the nerve trunk. Hematoxylin-eosin. Ob. 40. Oc. 10; д) plethora of vessels and hyperchromia of dendrites of the acoustic nerve. Hematoxylin-eosin. Ob. 8. Oc. 10; е) dystrophy of nerve cells of the temporal lobe of the brain with a microglial reaction. Hematoxylin-eosin. Ob. 40. Oc. 10.

Thus, the study of the effect of noise and vibration on the acoustic organ of animals showed that a hemodynamic disorder with morphological changes of the vascular wall and dystrophy of cellular structures was the common change for all its sections. Whereas a serous inflammation was detected in the middle ear along with hemodynamic disorders, dystrophic phenomena predominated in the inner ear. These changes were especially pronounced in the hair and sustentacular cells of Corti's organ, as well as in the neurons and dendrites of the spiral ganglion. The data obtained agree with the data by T. A. Tret'yakova and A. I. Pokotilenko (1976), which first showed that the vascular factor, that is, affection of vessels with a disturbance in microcirculation in the cochlea and subsequent dystrophic-destructive changes in nerve receptor elements, plays an important, if not the main, role in the pathogenesis of a

hearing disorder due to noise. Dystrophic phenomena are most pronounced along the trunk of the eighth nerve, as well as in the temporal lobes of the brain. Many cells were in a state of disintegration and a microglial reaction was also observed there.

Thus, on the basis of experimental investigations one can conclude that a prolonged effect of noise and vibration on the bodies of experimental animals produces gross changes in their acoustic organs.

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METHODS OF DETERMINING THE ECONOMIC EFFICIENCY OF INTRODUCTION OF SCIENTIFIC ACHIEVEMENTS IN OTOLARYNGOLOGY

Kiev ZHURNAL USHNYKH NOSOVYKH I GORLOVYKH BOLEZNEY in Russian No 3, 1978 pp 94-96

Article by T. Ye. Kolesnik, Candidate of Medical Sciences V. G. Kryzhanova, Candidate of Medical Sciences M. A. Perebatova, and Candidate of Economic Sciences T. I. Shchedrina, from the Scientific Organization Division (Candidate of Medical Sciences M. A. Perebatova, head) of the Kiev Scientific Research Institute of Otolaryngology (Doctor of Medical Sciences A. I. Tsyganov, director) and from the Sector of the Science of Science (Candidate of Economic Sciences T. I. Shchedrina, head) of the Institute of Cybernetics of the Ukrainian SSR Academy of Sciences (Academician V. M. Glushkov, director)

Text The problem of determining the social and economic efficiency of the results of scientific and practical activities of otolaryngologists was worked out by many researchers (A. I. Kolomiychenko, N. P. Feygin, M. V. Tarasyuk, M. A. Perebatova and Zh. G. Arkhangel'skaya, 1971; A. I. Kolomiychenko and V. A. Gukovich, 1974). The studies mentioned analyze the applied methods of calculating economic efficiency and propose original methods of calculation. Often, however, they are applicable only when a small number of applied problems are solved; for example, for the calculation of the social and economic effect of hearing improving operations.

The authors mentioned did not use the approach from the standpoint of the science of science encompassing the solution of a large number of theoretical and applied problems and making it possible from unified positions to classify and evaluate the efficiency of many stages in scientific development and introduction.

The above-stated impelled us to substantiate, develop and confirm the methods of calculating the economic effects obtained from the introduction of scientific research.

1. When including a subject in the plan, the executors calculate the assumed economic effect. This effect is not noted for a high accuracy, because the consequences of application of the new method of treatment are not yet known.

In principle, however, the achievement of an economic effect for medical scientific research institutes should not be an end in itself, but the consequence of application of their methods of treatment. This means that, when a scientific research subject is included in the plan, the extent of the assumed economic effect should not play the role of a decisive factor.

2. When a scientific research subject is fulfilled, the assumed economic effect is refined and is called expected or potential. The expected economic effect is the most objective indicator during an evaluation of the results of activity of a scientific research institute or another subdivision of a medical specialization, because the actual economic effect largely depends on the users applying a specific method of treatment.

3. The economic effects examined above are dynamic in their nature. In time the assumed economic effect is transformed into expected and the latter, into actual.

However, it is necessary to single out the conventional economic effect, which is of a one-time nature and greatly supplements both the expected and actual effect.

#### Methods of Calculating Economic Effects

1. The economic effect from the introduction of new methods of treatment into medical practice.

As a rule, the new method of treatment widely used in practice has a number of differently manifested economic effects. The most important of them (both actual and conventional) are presented in table 1.

Table 1.

In Sequence	Type of Effect	Conven. desig.	Actual, conven.	Period
A	Savings obtained by hospitals (polyclinics) when recovered patients are discharged ahead of schedule	ДВ ЭФ	Actual	
B	Economic effect obtained by society from a more rapid return of recovered patients to work	БТ ЭУЧР	Conventional	
C	Savings of the social insurance fund	ЭФС Ф	Actual	

A. The savings obtained by hospitals (polyclinics) when recovered patients are discharged ahead of schedule as a result of the application of the new method of treatment are calculated according to the following formula:

$$\mathcal{E}_{\Phi}^{\text{ПВ}} = (\Delta_1 - \Delta_2) \cdot C_{cp} \cdot A, \quad (1)$$

where  $\mathcal{E}_{\Phi}^{\text{ПВ}}$  is the actual economic effect from discharging a patient ahead of schedule;  $\Delta_1 - \Delta_2$  is the number of days of a patient's stay in the hospital with the old and new methods of treatment;  $C_{cp}$  is the average cost of 1 bed-day (rubles) in the hospital;  $A$  is the number of patients discharged from the hospital (polyclinic) ahead of schedule.

The specific nature of calculation of this effect lies in the fact that cured patients regardless of their age serve as the object for calculation.

B. The economic effect obtained by society when a new method of treatment, as a result of which a patient returns to socially useful work more rapidly, is introduced into practice is calculated according to the following formula:

$$\mathcal{E}_{\text{усл}}^{\text{ВТ}} = (\Delta_1 - \Delta_2) \cdot H_{\Delta} \cdot A^1, \quad (2)$$

where  $\mathcal{E}_{\text{усл}}^{\text{ВТ}}$  is the conventional economic effect from the return of a patient to work;  $\Delta_1 - \Delta_2$  is the number of days after which a patient returns to socially useful work with the old and new methods of treatment;  $H_{\Delta}$  is the national income created by one worker in 1 day (rubles);  $A$  is the total number of recovered patients cured by a given method who returned to work.

This economic effect is considered conventional, because both workers of the production and nonproduction spheres return to work. In principle, one could separate the actual effect from the total amount of the conventional effect, but, first, this is a very labor-intensive job connected with the division of recovered patients into two categories in accordance with their employment in the production and nonproduction spheres and, second, national income created only in the sphere of material production including industry, agriculture, construction, transport and communication, as well as the sphere of circulation, but, at the same time, also extending to the sphere of nonmaterial activity is the basis for the calculation of this effect.

C. The savings of the social insurance fund of enterprises, institutions and other social institutes are calculated according to the following formula:

$$\mathcal{E}_{\Phi}^{\text{ФС}} = \langle \Delta_1^{11} - \Delta_2^{11} \rangle \cdot C_{cp}^{\text{ВН}} \cdot A^{11},$$

where  $\mathcal{E}_{\Phi}^{\text{ФС}}$  is the actual economic effect as a result of the savings of the social insurance fund;  $\Delta_1^{11} - \Delta_2^{11}$  is the number of days granted to a patient on the basis of a temporary disability certificate with the old and new methods of treatment;  $C_{cp}^{\text{ВН}}$  is the average cost of 1 day of temporary disability (in the USSR, 4.6 rubles);  $A^{11}$  is the number of patients cured with a given new method of treatment who received reduced medical certificates of temporary disability.

Reference for practical calculations: 1) In the next few years one person employed in the USSR national economy will account for an average of 3,000 rubles of the national income annually (12.3 rubles per day); 2) 80 out of 100 patients are employed in the national economy; 3) pay for 1 day of temporary disability costs the state an average of 4.6 rubles; 4) there are 60 paid work days per 90 calendar days, that is, 243 days in a year.

The figures cited are tentative and are borrowed from the methodological data in "Aktual'nyye Voprosy Upravleniya Nauchnymi Issledovaniyami v Nauchno-Issledovatel'skikh i Meditsinskikh Institutakh Ministerstva Zdravookhraneniya RSFSR" [Urgent Problems of Management of Scientific Research in the Scientific Research and Medical Institutes of the RSFSR Ministry of Health], Moscow, 1974.

In the last few years ever greater attention has been given to agents speeding up repair processes during the postoperative period. In particular, for this purpose methyluracil was used in 13,000 patients who had tonsillectomy in the Ukraine during 1974-1976. At the same time, the period of stay in the hospital was shortened from 7.5 to 5.5 days.

The savings obtained by the hospital when recovered patients are discharged ahead of schedule as a result of the use of a new method of treatment total:

$$\mathcal{E}_{\Phi}^{\text{БН}} = (7.5-5.5) \cdot 6 \text{ rubles 03 kopecks} \cdot 13,000 = 156,780 \text{ rubles.}$$

The economic effect obtained by society when a given method of treatment, as a result of which a patient returns to socially useful work more rapidly, is introduced into practice is calculated only with regard to 8,000 adult patients and totals:

$$\mathcal{E}_{\text{усл}}^{\text{БТ}} (7.5-5.5) \cdot 12 \text{ rubles 03 kopecks} \cdot 6,400 = 153,984 \text{ rubles} \approx 154,000 \text{ rubles.}$$

The savings of the social insurance fund of enterprises, institutions and other social institutes are equal to 73,600 rubles:

$$\mathcal{E}_{\Phi}^{\text{ФС}} = (14-12) \cdot 4.6 \text{ rubles} \cdot 8,000 = 73,600 \text{ rubles.}$$

In the last 3 years the total economic effect from the introduction of methyluracil into practice in tonsillectomy has been 383,780 rubles.

D. The economic effect from the application of new methods of treatment of incurable patients returning to life: a) provided a patient returns to his previous work place:

$$\mathcal{E}_{\text{усл}}^{\text{БЖ}} = (57-5) \cdot \text{Н}_{\Delta} \cdot \text{A}, \quad (4)$$

where  $\mathcal{E}_{\text{УСЛ}}^{\text{БК}}$  is the conventional economic effect from the return of a patient to life; 57 is the average age of retirement on a pension; B is the age of an able-bodied incurable patient;  $N_{\text{Д}}$  is the average annual national income per person employed in the USSR national economy; A is the total number of patients who returned to life; b) provided a patient returns to socially useful work connected with retraining:

$$\mathcal{E}_{\text{УСЛ}}^{\text{БК}(\Pi)} = (57-B) \cdot N_{\text{Д}} \cdot 0.5 \cdot A, \quad (5)$$

where 0.5 is the correction coefficient taking retraining into account.

A mean value is accepted, because the information necessary for its accurate calculation is not available. If the age of such a patient is below 18, the amount (B) assumes the value 18.

E. The economic effect from the application of new methods of treatment prolonging the life of incurable patients:

$$\mathcal{E}_{\text{УСЛ}}^{\text{ПД}} = \Pi_{\text{пр.}} \cdot N_{\text{Д}} \cdot A, \quad (6)$$

where  $\Pi_{\text{пр.}}$  is the number of prolonged years of life of an incurable patient who returned to socially useful work; A is the number of these types of patients.

2. The economic effect from the application of new methods of diagnosis in medical practice.

This effect is manifested when the number of errors during the diagnosis of a specific disease "X" is reduced. Since, as a rule, wrong diagnoses involve economic losses (increase in the period of cure of a patient in the hospital or polyclinic) and, accordingly, a reduction in his labor contribution to public production, prevention of these economic losses expressed in rubles will be the economic effect.

The general formula of the determination of this effect appears as follows:

$$\mathcal{E}^{\text{МД}} = \Pi^{\text{Э}} (K_1^{\text{од}} - K_2^{\text{од}}), \quad (7)$$

where  $\Pi^{\text{Э}}$  are the prevented economic losses per wrong diagnosis;  $K_1^{\text{од}}$ ,  $K_2^{\text{од}}$  is the number of wrong diagnoses per 100 cases with the old and new methods of diagnosis.

The value ( $\mathcal{E}^{\text{МД}}$ ) can be both actual and conventional. It depends on the specific nature of calculation of the value ( $\Pi^{\text{Э}}$ ). If ( $\Pi^{\text{Э}}$ ) is calculated according to the increase in the period of a patient's stay in the hospital (polyclinic), the economic effect ( $\mathcal{E}^{\text{МД}}$ ) is actual. If economic losses are calculated according to the reduction in the labor contribution of patients discharged later into public production, the economic effect ( $\mathcal{E}^{\text{МД}}$ ) is conventional.

These effects should be calculated according to formulas (1) and (2) of this work.

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TRICHOMONAS PRESENCE IN ORAL AND NASAL CAVITY AND IN EARS

Kiev ZHURNAL USHNYKH NOSOVYKH I GORLOVYKH BOLEZNEY in Russian No 3, 1978  
pp 97-98

Article by L. V. Trofinova, E. K. Siyrde and Kh. P. Lentsner, from the Department of Otorhinolaryngology (Prof E. K. Siyrde, consultant) and the Sector of Microbiology (Candidate of Medical Sciences Kh. P. Lentsner, head) of the Central Scientific Research Laboratory of Tartu State University/

Text More than 100 species of parasitic Flagellata from the family Trichomonadidae are known. Most of them are parasites of birds and mammals. Man is the host of three Trichomonas species (Trichomonas tenax, Trichomonas vaginalis and Trichomonas hominis).

Trichomonas tenax is often detected in various diseases of the oral cavity. There are data that in the above-mentioned pathology among the population of North Italy they are detected in 43% (Carneri and Gionnone, 1965), in the Azerbaydzhan SSR, in 62% (F. A. Musayev, 1969) and in some regions of Yugoslavia, even in 75% (Simitch, 1957). This Trichomonas species is detected in the nasal cavity, tonsils and lungs. However, very little is known about the pathogenicity of this protozoon and about its etiological role in the pathology of the ear and the oral and nasal cavity. This is connected with the absence at the present time of a suitable model for the study of the pathogenicity of T. Tenax, because it is very difficult to obtain axenic cultures of this species in connection with the diversity of the microflora of the ear and the oral and nasal cavity. Not a single bibliographic source considers T. Tenax a primary etiological factor in the pathology of the oral cavity.

Many authors believe that the frequency of detection of T. tenax largely depends on the hygienic state of the oral cavity. T. tenax is often detected in the oral cavity in individuals with dental caries and gingivitis (Ruiz, 1958).

In the Estonian SSR a group of scientists under the guidance of Prof Yu. Kh. Teras (Institute of Experimental Biology of the Estonian SSR Academy of Sciences) are engaged in the study of the etiological role of T. tenax in the

onset of the diseases of the oral cavity and respiratory tracts. Studying the possibilities of obtaining axenic cultures of *T. tenax*, they detected a very high sensitivity of this *Trichomonas* species to a change in the medium composition. It was possible to cultivate *T. tenax* for three or four passages on Locke's modified medium--a new liquid phase covering the dense base of Locke's egg-serum medium (Yu. Kh. Teras, Kh. Ya. Tempel', E. A. Mirme and E. V. Kallas, 1970). The above-mentioned authors expressed the opinion that *Trichomonas*, as frequent companions of purulent diseases of the bronchi and lungs, can greatly affect the course of pathological processes in these organs.

We did not find data in the literature on investigations of *T. tenax* from an otorhinolaryngological aspect. According to the data by Teisanu (1973), only in five individuals with purulent highmoritis *T. vaginalis* was found in the discharge from the nose and in the urogenital tract. Recovery came after a specific treatment.

In the Department of Otorhinolaryngology of the Tartu Republic Clinical Hospital we examined the effect of *T. tenax* on the pathological processes in the ear and in the oral and nasal cavity. We examined 90 patients (55 women and 35 men) with the following diagnoses: chronic purulent highmoritis, 18; acute purulent inflammation of the middle ear, 11; acute purulent recurring inflammation of the middle ear, 3; chronic purulent inflammation of the middle ear, 20; chronic tonsillitis, 19; chronic purulent inflammation of the frontal sinus, 3; abscess of the nasal septum, 1; purulent cervical lymphadenitis, 1; burn of the oral, pharyngeal and esophageal cavity with caustic substances, 7; allergic rhinosinusopathy, 2; acute mastoiditis, 1; cyst of the maxillary sinus, 2; purulent rhinitis, 2. The age of the patients was from 1 year to 78 years, most of them being 30 to 39 years old.

As the material for examination we took the deposit from the gingiva, the purulent content of the cavity of the abscess of the nasal septum, the fluid of the cyst of the maxillary sinuses, the discharge from the nasal cavity, from the ears and from the accessory sinuses of the nose and tonsillar pus. If the process was bilateral, we took the material from both inflammation foci.

Of the 31 cases of purulent inflammation of the middle ear (both acute and chronic) in 27 cases the purulent discharge from the ears and the deposit from the gingiva were examined simultaneously. In seven observations of the purulent inflammation of the middle ear it was possible to take the material twice--at the initial stage of the disease and at the remission stage, when the purulent discharge was detected in a minimum amount. In burns with caustic substances the deposit from the gingiva was also taken twice--at the stage of necrosis and at the stage of epithelization.

Immediately after the material was taken, we prepared the native preparation in a physiological solution and examined it under a microscope. Actively moving *Trichomonas* were seen in the preparation in positive cases. In

each positive observation two or three drops of the material were inoculated with Pasteur's pipet on Locke's egg-serum medium (Jollos, 1930). After the inoculation 1,000 active units of penicillin per ml of the medium were added to the medium. The media were checked daily after the inoculation for 1 week.

In 31 out of the 90 cases Trichomonas were detected in the native preparation. *T. tenax* was detected in three people with an acute purulent recurrent inflammation of the middle ear, in three people with an acute purulent inflammation of the middle ear, in seven people with a chronic purulent inflammation of the middle ear, in three people with chronic tonsillitis, in one person with a chronic purulent inflammation of the frontal sinus, in one person with purulent cervical lymphadenitis, in seven people with a burn of the oral, pharyngeal and esophageal cavity with caustic substances, in one person with acute mastoiditis, in two people with purulent rhinitis and in two people with chronic purulent highmoritis.

In individuals with inflammation of the middle ear (both acute and chronic), with acute mastoiditis and with chronic tonsillitis, in whom Trichomonas were detected in the discharge from the ears and in tonsillar pus, Trichomonas were also detected in the deposit from the gingiva. Only four people had dental caries.

In all the seven patients suffering from a chronic purulent inflammation of the middle ear a repeated examination of the material after treatment for Trichomonas presence produced a negative result.

Examination of the material in burns with caustic substances at the stage of epithelization also produced a negative result in all cases. It should be stressed that in burns with caustic substances, when a chemical factor was the cause, *T. tenax* was found only during the necrotic phase.

When the material was inoculated on Locke's egg-serum medium, Trichomonas could not be grown.

We examined a control group of 14 people, in whom pathology in the ears and in the oral and nasal cavity was not noted. In this group the discharge from the nose and the deposit from the gingiva simultaneously served as the examination material. Trichomonas were not detected in any of them, neither in the native preparation, nor during inoculation on Locke's egg-serum medium.

In 35 out of the 90 patients the pH of the material was determined. *T. tenax* was detected in the material with pH from 6.6 to 8.1. When Trichomonas disappeared from the material, the pH was changed in the alkaline direction.

The acid medium is a more favorable habitat for *T. tenax*. In addition to Trichomonas, streptococci, staphylococci, Sarcina and various types of fungi were detected in the material (treatment with ordinary anti-inflammatory methods).

Thus, Trichomonas were not detected in people without pathology of the ears or of the oral and nasal cavity. In case of recovery after burns with caustic substances and inflammations of the middle ear Trichomonas disappeared.

The data of our investigations show the following.

In one-third of the observations of various pathological states *T. tenax* were found in the ear pus, in the pus from the accessory sinuses of the nose and even in the pus from cervical lymph nodes. All this indicates that Trichomonas are not only in the oral cavity, but move together with various pyogenic bacteria in organs that are the focus of inflammation. *T. tenax* are the indicator of the hygienic state of the oral cavity, as described in the literature.

On the basis of the data obtained we cannot yet say what role *T. tenax* plays in the onset and course of the inflammatory process in otorhinolaryngological organs.

However, the observations presented point to the need to examine, along with the pathogenic microflora, the presence of *T. tenax* in the oral cavity and in the discharge from the nose and ears.

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## VETERINARY MEDICINE

### SANITATION MEASURES IMPROVED ON FARMS

Moscow VETERINARIYA in Russian No 6, 1978 pp 3-5

[Article: "Sanitation Measures on Farms"]

[Text] The workers of livestock breeding farms, just as all the workers in the country, have joined actively in the competition to fulfill and overfulfill the state plans and raise production efficiency and work quality. To make the third year of the five-year plan a year of all-out effort to achieve new progress in implementing the resolutions of the 25th CPSU Congress-- that is the goal of the sector's workers.

Reinforcing the material base, specialization and mechanization of the farms, introduction of advanced technology and an increase in the skills of the personnel are creating a sound basis for a further upsurge in livestock breeding. Last year at the kolkhozes and sovkhozes the sale of livestock and poultry for meat rose by almost 10 percent. The increase in milk production was 5.1 million tons. Some 5.2 billion more eggs were obtained than last year. The stock noticeably increased and the productivity of the herd increased. Now it is important to secure what has been attained and to ensure a further growth in the output of products.

In this respect, summer is the most favorable time, when the farm workers are striving to give the country more milk and meat of better quality and to make fuller use of the potentials of the pasturage season. The outstanding collectives have organized a broad search for potentials, are intensively grazing and fattening the livestock, using inexpensive green fodders, are increasing the milk yield and weight gains and increasing the stock.

In the summer the veterinary specialists are called upon to organize preventive medical measures correctly and efficiently. It is important to avert livestock diseases, to create the optimal animal health care conditions for feeding and maintaining the animals on the farms and to ensure high sanitation standards.

Under the conditions of concentration and intensification of livestock breeding, sanitation measures have become a compulsory component of the industrial production process. Achievements in veterinary sanitation have

made it possible, in accordance with scientifically substantiated norms, to plan and construct livestock breeding facilities at kolkhozes and sovkhozes, which ensures a high sanitation standard and optimum conditions for keeping the livestock and poultry and makes it possible to produce high-quality goods.

The farms and complexes are planned in accordance with the existing industrial norms. In addition, all the standard and experimental plans worked out undergo expert examination at veterinary scientific research institutions and organs of the state veterinary service.

The specialists of the Main Administration of Veterinary Science of the Ministry of Agriculture of the Moldavian SSR are paying great attention to the construction of livestock breeding farms in accordance with the standard plans. At all the new livestock breeding farms and complexes they supervise the construction of the veterinary-sanitation facilities, including sanitation and preventive disinfection establishments, steam-Formalin chambers, rooms for changing clothing and disinfection barriers for processing motor vehicle transport and footwear.

Not all the veterinary organs and operating specialists, however, regularly supervise adherence to the veterinary requirements in the planning, construction and renovation of livestock breeding facilities.

The errors made by the planners and construction engineers are difficult to eliminate when the farms and complexes are in operation, and lead to a considerable economic loss as the result of the reduction in the animals' productivity, their diseases and epizootic.

When a dairy complex for 1200 cows was turned over for operation at the Bobrovskiy Sovkhoz in Voronezhskaya Oblast, the commission did not direct attention to the quality of the floors, and as a result a large number of hoof injuries were recorded, and the incorrect design of the feeder limiters prevented the animals from eating the fodder normally and caused injuries to their necks and heads.

At the Kuban' Sovkhoz in Krasnodarskiy Kray, at a complex for 1200 cows, the dairy unit was constructed at a distance of over 40 meters from the milking room, which made the milk line longer, made its sanitary processing more complicated and affected the quality of the milk.

A successful solution to the problems of disinfection and efficient use of manure contributes to raising the sanitation standard at livestock breeding farms and reduces the probability of environmental pollution. The importance of this problem is particularly obvious if one considers that the kolkhozes and sovkhozes yearly apply to the soil over 600 million tons of organic fertilizers, and about 150 million tons are not used, remain on the farms and lead to pollution of the facilities and territory of the farms. A situation that is particularly unfavorable in this respect is developing at the farms of the Kazakh SSR and a number of other republics, krays and

oblats. Specialists of the state veterinary service must work out measures, in conjunction with the agricultural organs, for yearly complete and high-quality ridding the farms of manure, its disinfection and efficient use.

A great deal of attention is now being paid to utilizing manure at industrial livestock breeding complexes. A great deal has been done in this respect by scientific research planning institutes, but many problems still remain unsolved. This leads to the existing facilities for disinfecting manure as a rule doing unsatisfactory work, as the result of which the environment is polluted. The main reasons for the poor work of the purification installations at the Gornoural'skiy Hog Breeding Complex in Sverdlovskaya Oblast, the Borisovskiy in the Belorussian SSR, imeni 50-Letiye SSR in Gor'kovskaya Oblast and a number of others are excess entry, against that planned, of sewage from the industrial zones, lack of correspondence of the overflow trough-settling tanks and inadequate enrichment of the sewage with oxygen in the aeration tanks due to imperfection in the design.

At modern industrial complexes on limited territories, a large number of animals are concentrated, and therefore any violation of the regulations for building up the herd leads to undesirable results. For example, at the Sovkhoz imeni Frunze in Issyk-Kul'skiy Rayon in the Kirgiz SSR, 137 cows were brought and supplied to the complex from a farm that was unsafe with respect to brucellosis, as the result of which the healthy pedigreed livestock of the complex was infected with brucellosis.

To raise the sanitation standard on the farms and obtain high-quality products, great importance is attributed to holding a regular sanitation day. With the introduction of a number of organizational-farm measures, this to a considerable extent predetermines the farm's high production indicators.

For example, at the dairy farms in Voronezhskaya Oblast the veterinary specialists, in conjunction with the zootechnicians and farm directors, are daily seeking new ways to improve the quality of the livestock breeding products obtained, are creating good labor conditions for the farm workers, train them constantly, and have developed socialist competition to the motto, "For the Products of the Farms--Guaranteed Quality." As the result of these measures, most of the farms in the oblast sell mainly first class dairy products to the state. In Rostovskaya Oblast a competition was organized for awarding the Seal of Quality to the milk turned over.

The dairy farm of the Ala-Arche Sovkhoz in Alamedinskiy Rayon in the Kirgiz SSR is distinguished by a high standard of feeding and maintaining the livestock and the good sanitary condition of the facilities and milking equipment. The milkers and cattle tenders are always dressed in clean overalls. It is not surprising, therefore, that here there are no infectious diseases of the animals nor murrain among the young stock. Only first class products are received from here. A great deal of credit for this goes to the veterinary service headed by S.Ye. Komnatnyy. He helps the farm collective to achieve outstanding milk yields and a high issue of calves.

On some farms in the Kazakh SSR, however, the directors and veterinary specialists do not pay the proper attention to these problems. They do not create normal conditions for the primary processing of the milk on the farms, do not supervise the sanitary condition of the milking and the dairy equipment and do not construct auxiliary facilities. This attitude toward the matter affects the reduction in quality of the dairy products obtained. In 1977 the republic's farms sold the state about 50 percent non-graded milk.

Preventive disinfection, disinfestation, insect extermination and rat extermination are exceptionally important in the system of veterinary-sanitation measures in livestock breeding. A large number of animals and millions of square meters of facilities are subjected to these treatments. There is everything necessary to carry out this work successfully--trained personnel, disinfectant agents and modern disinfection equipment. The veterinary organs now have 40,000 disinfection units and industry yearly supplies over 60,000 tons of disinfection and disinfestation agents. Their correct use is the primary duty of veterinary specialists. Despite this, there are cases when the disinfection agents are stored outdoors, spoil, no record is made of their consumption and the portable disinfection equipment is not used according to its purpose.

In order to improve the veterinary-sanitation conditions of livestock breeding farms, raise the quality of the products and introduce planned implementation of disinfection and other prophylactic work in a number of republics, krays and oblasts, economically accountable veterinary-sanitation detachments have been established.

Work experience shows that in the area of their work there is a sharp improvement in the epizootic state of the livestock breeding, the veterinary and sanitation standard of the farms is higher and the disinfection agents and disinfection equipment are efficiently and effectively used. These detachments are a school for the training of skilled disinfectant and rat extermination workers.

Now, when the transfer of livestock to pasture maintenance has been completed everywhere, the time has come to carry out the prophylactic sanitation repair of the livestock breeding facilities. Where these measures are specified by the industrial finance plan, this work should be completed on schedule. At farms where they are planned, the veterinary specialists, in conjunction with the directors, must seek means to carry out the sanitary repair of the farms. This is particularly true of farms that are unsafe with respect to chronic infectious and formerly unsafe with respect to highly infectious diseases of the animals. Work should be organized so that each livestock breeding facility is subjected to the necessary sanitation repair, compulsorily--disinfection and whitewashing--and the grounds of the farms rid of manure. Carrying out these measures will serve as a pledge to improving the veterinary-sanitation standard and epizootic state of the farms, raising the sanitary quality of the products obtained and successful carrying out of the coming wintering of the livestock.

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## SCIENTISTS AND SCIENTIFIC ORGANIZATIONS

### SYMPOSIUM ON MYCOTOXINS

Kiev MIKROBIOLOGICHESKIY ZHURNAL in Russian No 3, 1978 pp 394-396

[Article by A. M. Zaychenko and Z. A. Kurbatskaya]

[Text] There was a regional symposium in Orenburg, from 28 to 30 September 1977, dealing with mycotoxins, entitled "Mycotoxins, Producers, Chemistry, Biosynthesis, Demonstration [or Definition] and Effects on the Organism," which was convoked by the Orenburg Medical Institute, Institute of Nutrition of the USSR AMS [Academy of Medical Sciences], Institute of Microbiology and Virology, Ukrainian AS [Academy of Sciences], All-Union Scientific Research Institute of Experimental Veterinary Science, All-Union Scientific Research Institute of Grain and Grain Products and All-Union Scientific Research Institute of Veterinary Sanitation, with the active support and participation of the Executive Committee of the Orenburgskaya Oblast Council of Workers' Deputies, oblast council of NTO [Scientific and Technical Society], oblast board of the All-Union Chemical Society imeni D. I. Mendeleyev and oblast board of the NTO of the food industry.

It was noteworthy for the symposium that it was dedicated to the 60th anniversary of the Great October Revolution.

A total of 550 people participated in the work of the symposium; they represented 29 scientific research, educational and departmental institutions, as well as workers from 18 cities of the Soviet Union. Among them were one corresponding member of the Ukrainian AS, 3 honored scientists, 22 doctors of sciences and 69 candidates of sciences.

In accordance with the agenda of the symposium, 78 papers, 24 of which involved the use of displays, were delivered dealing with pressing problems of mycotoxicology.

Specialists in varying fields were concerned with the problem of mycotoxins: mycologists, microbiologists, medical and veterinary physicians, chemists, hygienists, workers in the food industry and agriculture.

T. Z. Boyarshinova, chairperson of the symposium organizing committee and deputy chairperson of the Orenburg oblispolkom, and Prof Z. K. Zabel'skaya, pro-vice-chancellor for scientific work at Orenburg State Medical Institute, delivered the opening remarks and welcomed the participants at the symposium.

The following individuals delivered scheduled papers dealing with the most pressing problems of mycotoxicology and objectives of continued development of this discipline: Prof A. Kh. Sarkisov, doctor of biological sciences (All-Union Scientific Research Institute of Experimental Veterinary Science, Moscow); Prof V. I. Bilay (Institute of Microbiology and Virology, Ukrainian AS, Kiev), doctor of biological sciences; M. A. Akhmeteli, doctor of medical sciences (Institute of Epidemiology and Microbiology imeni N. F. Gamaleya, Moscow); Prof L. Ye. Olifson, doctor of biological sciences (Orenburg State Medical Institute, Orenburg); Prof Ye. P. Kozhevnikova, doctor of medical sciences (Orenburg State Medical Institute, Orenburg), and V. A. Tutel'yan, candidate of medical sciences (Institute of Nutrition, USSR AMS, Moscow).

In his paper, A. Kh. Sarkisov praised highly the contribution of the Ukrainian school of mycotoxicologists to development of this discipline in our country; he dwelled on the main elements of the ecological classification of mycotoxicoses, the significance of potential hazard of many mycotoxins, difficulties involved in isolating and identifying them, the need to develop standardized simple and reliable indication methods. This speaker also called attention to the need to create a quarantine service to control [inspect] imported grain products and forage, as well as expansion of VUZ programs [or syllabuses] dealing with mycology and mycotoxicology.

V. I. Bilay discussed comprehensively the mycological aspects of the problem, which are the leading ones in the problem as a whole. This speaker shed light on questions of systematics of toxin-producing fungi. A significant place was devoted to the problem of detoxification of mycotoxins, physiological and biochemical distinctions of producer fungi as related to bio-synthesis of mycotoxins. V. I. Bilay mentioned the need for continued investigation of physiology of toxin synthesis and toxic properties of metabolites in the ecological and geographic aspect, their chemistry and biological effects. She also discussed some organizational problems and, in particular, the need to create a coordinating center for this problem.

The paper of M. A. Akhmeteli dealt with epidemiological aspects of the problem of mycotoxicosis, as well as pathogenesis of diverse forms thereof. This speaker, who used excellent statistical material, demonstrated convincingly the territorial, climatic, age-related and other differences in onset and distribution of some forms of mycotoxicosis. Specific patterns inherent in mycotoxins were demonstrated on the basis of epidemiological studies of endemic nephropathy and primary cancer of the liver.

In his paper, L. Ye. Olifson remarked that the sterols formed by *Fusarium* *sporotrichiella* Bilai play the leading role in survival of fungal cells under adverse conditions. He also mentioned the possible biogenetic correlation between sterols and trichothecial mycotoxins.

Pathomorphological changes in organs and tissues of experimental animals, which proved the carcinogenic effects of sporofusarin, were described in the paper of Ye. P. Kozhevnikova.

V. A. Tutel'yan observed that, at the present time, it is probably not enough to explain the carcinogenic effects of mycotoxins, particularly aflatoxins, by relating them to DNA and consequent inhibition of protein and nucleic acid synthesis. Several facts were obtained as a result of many years of work in the laboratory headed by A. A. Pokrovskiy, which permit classification of mycotoxins in the group of so-called membranotoxins with marked lysosomal tropism.

There were three sections at the symposium: 1) Producers, chemistry, bio-synthesis of mycotoxins. 2) Effects of mycotoxins on the organism. 3) Methods of demonstrating mycotoxins.

The papers delivered in the first section reported on the physiological distinctions of toxin production in some fungi, in particular in representatives of the genera *Fusarium*, *Dendrodochium* and *Aspergillus* (papers delivered by V. I. Bilay, A. M. Zaychenko, Z. A. Kurbatskaya, Kiev; N. A. Kostyunina, Moscow), methods of obtaining, means of biosynthesis and chemistry of different mycotoxins (M. Goshayev et al., Ashkhabad; T. Ye. Tolcheyeva et al., Orenburg; V. V. Yermakov et al., Moscow; K. P. Panozishvili, A. V. Borovkov, Leningrad) and biological effects (V. V. Semenov et al., Kazan'; G. I. Borodin et al., Tashkent).

A considerable place in this section was referable to papers dealing with the distribution of potentially toxigenic and toxigenic species of fungi on feed and foodstuffs, as well as in modeling of processes of self-heating of grain (A. S. Bukharbayeva, P. S. Nikov; P. S. Nikov et al.; L. M. Fadeyeva et al., Alma-Ata; N. I. Sosedov, A. P. Shul'gina et al., Moscow; L. I. Lozbin et al., Kiev).

The paper of V. A. Trufanova and A. N. Kotik (Borki Station, YuZhD [Southern Railroad?] evoked special interest; it dealt with the study of virus-like particles in the mycelium of a toxigenic strain of *Fusarium sporotrichiella* and determination of their possible role in toxinogenetic processes.

In the section entitled "Effects of Mycotoxins on the Organism," several papers were delivered on the symptomatology of mycotoxicosis in farm animals (I. S. Yelistratov et al., Omsk; A. N. Kotik, V. A. Trufanova, Borki Station, YuZhD; V. V. Rukhlyada, S. M. Nikolayev, Kirovograd), as well as histomorphological and histochemical changes in the presence of experimental mycotoxicosis (S. M. Gubkin, L. I. Il'ina; P. A. Il'in, A. M. Kogan, Omsk).

The section entitled "Methods of Demonstrating Mycotoxins" was of considerable interest to the participants of the symposium and particularly practical workers. It was shown that modern mycotoxicology has a number of physico-chemical and biological methods of identifying mycotoxins (O. V. Bukharin et al.; K. G. Galkovich et al., Orenburg; V. V. Yermakov; A. N. Leonov, O. V. Chizhova; V. P. Nelyubin et al.; Yu. I. Boikov, E. M. Khasanova, Moscow; R. G. Ibragimov, Baku). The speakers reported that detection of mycotoxins in feed and foodstuffs requires the use of both chemical and biological methods; only combined use thereof can yield reliable results.

The symposium worked on a high scientific and organizational level, with active discussion of several issues. There was also an extensive program of display-related reports which proceeded concurrently with the work of the relevant sections.

The resolution adopted by the symposium at the concluding session noted the significant expansion of research on mycotoxins in the USSR, which is of theoretical and sanitary-hygienic significance to medicine, veterinary science, agriculture and other fields. In addition, the participants also noted the substantial flaws with respect to solving this problem. The most important ones are the lack of coordination of research on mycotoxins, lack of standardized methods of inspecting the quality of grain and other food products stricken by toxigenic strains of micromycetes, lack of recommendations for the use of stricken grain in the animal industry, etc.

On the basis of the critical speeches and comments, the editorial council of the symposium, which included some prominent scientists, elaborated a plan of measures dealing with the topics of scientific research and organizational matters, which was unanimously approved by the participants of the symposium.

The symposium deems it mandatory to request that the Presidium of the USSR AS create an interdepartmental commission under the department of biological sciences, USSR AS, dealing with mycotoxicology for the coordination of research in this field and development of state-level legislation, as well as creation of coordinating centers dealing with general problems of mycology and toxicology (Institute of Microbiology and Virology, Ukrainian AS, Kiev, and All-Union Scientific Research Institute of Experimental Veterinary Science, Moscow), for sanitary and hygienic evaluation of the quality of feed and food products (All-Union Scientific Research Institute of Grain and Grain Products and All-Union Scientific Research Institute of Veterinary Sanitation, Moscow), problems of epidemiology of mycotoxicosis (Institute of Epidemiology and Microbiology imeni N. F. Gamaleya, Moscow) and problems of mechanisms of biochemical action of mycotoxins (Institute of Nutrition, USSR AMS).

Summaries of papers delivered at this symposium have been published.

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## SCIENTISTS AND SCIENTIFIC ORGANIZATIONS

### PROBLEMS OF BACTERIOSIS DISCUSSED AT THE FIRST ALL-UNION CONFERENCE ON IMMUNITY OF VEGETABLES

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[Article by R. I. Gvozdyak]

[Text] In spite of their brilliant organization, conferences dealing with immunity of plants, which convened in prior years in Moscow, Kishinev and Kiev, revealed that it was inexpedient to hold such conferences to discuss immunity of all agricultural crops. The wide range of issues discussed and the large number of participants minimized the effectiveness of these forums of scientists. For this reason, it was decided to hold conferences dealing with different groups of agricultural plants.

The first such conference, dealing with immunity of vegetables, was organized by the All-Union Scientific Research Institute of Plant Growing imeni N. I. Vavilov, and it convened in Leningrad from 29 September to 2 October 1977. Among the participants, there were 4 academicians, 20 doctors, 55 candidates of sciences; in all there were about 100 people from 26 scientific institutions of our country. There were many phytopathologists and breeding specialists, and this determined the specificity and direction of the conference.

A total of 60 papers was delivered. Many of them touched upon general problems of immunity (M. S. Dunin, N. Ya. Dorozhkin, N. N. Zaginaylo). Particular attention was devoted to the methods of phytopathological evaluation of resistance [stability] of the collection of vegetables (E. A. Vlasova and others). The participants of the conference were shown an express method of determining resistance of cut leaves and seedlings of tomatoes and wheat.

As noted in the paper of V. I. Krivchenko, there is a large supply in the USSR of different cultigens, determination of the resistance of which to various diseases is far from completed. Moreover, the genetic resources could be augmented by using various mutagenic factors, for example,  $\gamma$ -rays and  $^{60}\text{Co}$  (V. V. Khrustalev, V. K. Shcherbinov).

In our analysis of papers delivered at this conference, we shall concentrate primarily on those dealing with bacterial diseases and their pathogens.

In his paper, R. I. Gvozdyak (Institute of Microbiology and Virology, Ukrainian Academy of Sciences (IMV, UkAS]) stated that in experimental determination of resistance one must take into consideration circadian rhythms, which determine the extent of plant susceptibility to bacterial infection. M. S. Matyshevskaya (IMV, UkAS) submitted experimental and literature data concerning induction by phytopathogenic bacteria of a supersensitive reaction in plants, the effects on it of various environmental factors and possible use of this reaction in breeding plants for resistance to bacterial diseases. Z. V. Knyazeva (All-Union Scientific Research Institute of Plant Protection) discussed the methodological procedures used in demonstration of resistance to the pathogen of bacterial blight in different varieties of tomatoes. Many papers discussed the deleteriousness of bacteriosis and need to breed plants resistant to it. Thus, V. I. Pozhenkov cited data on the significant harm inflicted by the pathogen of angular blight of cucumbers in many countries and the incidence of this bacteriosis in Moscow Oblast. The same bacteriosis caused perceptible loss of cucumber harvest in Moldavia (A. P. Khor'kov, N. M. Rudenko). The paper of A. V. Medvedev and N. I. Medvedeva submitted some interesting data on resistance to *Pseudomonas lachrymas* of a wide assortment of strains and breeding material in various geographic zones. Bacterial canker causes great loss of tomato harvest in Armenia (A. A. Babayan, S. A. Arapetova, S. M. Petrosyan). Bacterial leaf blight of tomatoes is widespread in Voronezhskaya Oblast (Z. V. Knyazeva), the Volga-Aktyubinsk floodplain (N. S. Kokareva). The time and conditions enhancing appearance of this bacteriosis in Krymsk were defined (G. F. Govorova). Vascular and mucous bacteriosis is dangerous to cabbage (A. A. Kazakova, Ye. T. Neklyudova). Unfortunately, some authors do not name the strains and races that they investigated.

The papers delivered were indicative of the need to pursue breeding work to obtain resistance to the set of the most harmful pathogens. As noted by Academician M. S. Dunin, this is a very complex task, the fulfillment of which requires the attention of highly qualified specialists and much time. However, it is a solvable problem, since researchers have observed, in a number of instances, a direct correlation between resistance to the pathogens of various diseases. Thus, cucumber resistance to the pathogen of bacterial angular blight is related to their resistance to powdery mildew (A. P. Khar'kova, N. M. Rudenko), powdery mildew and pernosporiosis (A. V. Medvedev, N. I. Medvedeva) and olive disease (V. I. Pyzhenkova). Tomato resistance to bacterial leaf blight is related to resistance to septoriosis and macrosporiosis (N. S. Kardayeva, L. N. Popova). Such a correlation between resistance to pathogens of different diseases must be particularly checked in conducting breeding work.

Breeding highly resistant varieties is the only means of significantly reducing the losses of harvest, without the use of chemical protective agents. The latter, as we know, are an important source of environmental pollution. The use of induced immunity may play a rather important role in lowering the use of toxic chemicals (M. S. Dunin, N. A. Dorozhkin, Yu. I. Vlasov). Replacement or at least partial reduction in the amount of toxic chemicals that are used is also important because some of them can be converted by a plant into compounds that are very hazardous to man (E. I. Slepyan).

Of interest are data indicating that the extent of varietal resistance is determined by the geographic origin of both the plant and pathogen. There have been some timely studies dealing with the effects of man's conquest of nature on the change in pathogens of diseases (D. D. Brezhnev, V. V. Farber). N. N. Zaginaylo stressed the fact that, when conducting breeding work, it is important to know the etiology of the disease and biology of the pathogen.

A resolution adopted by the conference mentioned the need to convoke similar congresses of scientists in the future, once every 3-4 years, as well as that the proceedings of this conference should be published, with annual publication of a bulletin reflecting data on resistance of plant varieties to infectious diseases in different geographic zones of the USSR.

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